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STUDIES ON EXPERIMENTAL PNEUMONIA.

V. Active Immunity against Experimental Pneumococcus Pneumonia in Monkeys Following Vaccination with Living Cultures of Pneumococcus.

By RUSSELL L. CECIL, M.D., and FRANCIS G. BLAKE, M.D.

(*From the Bacteriological Laboratory of the Army Medical School, Washington.*)

(Received for publication, January 23, 1920.)

In a preceding paper[1] it was shown that the subcutaneous injection of killed pneumococci in doses comparable to those employed in man will not protect monkeys against pneumococcus pneumonia of homologous or heterologous type. The aim of the present study has been to determine the prophylactic value of a vaccine composed of living pneumococci.

The conviction, based on considerable experimental evidence, has long been held by immunologists that a living virus stimulates a more effective resistance to infection than a killed virus.

Metchnikoff and Besredka,[2] in their studies on typhoid vaccination in the chimpanzee, found that very little protection was conferred by vaccination with dead typhoid bacilli, whereas animals vaccinated with small amounts of living cultures were efficiently protected. Haffkine's[3] method of vaccination against cholera consisted in the injection of 0.2 to 0.05 cc. of living culture of the cholera vibrio, first an attenuated culture being used and 5 days later a more virulent one. As early as 1893 Haffkine and his coworkers vaccinated 40,000 people in India by this method and obtained, on the whole, encouraging results. Kolle and Otto,[4] after experiments upon monkeys and other animals, concluded that dead plague bacilli were much inferior to attenuated living cultures for prophylactic vaccination.

[1] Cecil, R. L., and Blake, F. G., *J. Exp. Med.*, 1920, xxxi, 519.

[2] Metchnikoff, E., and Besredka, A., *Ann. Inst. Pasteur*, 1911, xxv, 193; 1913, xxvii, 597.

[3] Haffkine, W. M., *Bull. Inst. Pasteur*, 1906, iv, 825.

[4] Kolle, W., and Otto, R., *Deutsch. med. Woch.*, 1903, xxix, 493; *Z. Hyg. u. Infectionskrankh.*, 1903, xlv, 507.

Strong[5] made a thorough study of the various methods of plague vaccination and concluded that the most efficient method was immunization with attenuated living cultures. He showed that when carefully done this method can be employed in human beings.

The investigations referred to above indicate that for the diseases of bacillary origin at least, living vaccines are in many ways preferable to dead cultures.

The experiments included in this study may be divided into two groups: (1) vaccination with a living virulent culture of Pneumococcus Type I, and (2) vaccination with a living avirulent culture of Pneumococcus Type I.

EXPERIMENTAL.

Two species of monkeys were used in this investigation, *Cebus capucinus* and *Macacus syrichtus*.

Method of Vaccination.—The inoculations were administered subcutaneously in the abdominal wall. In the experiments with the virulent culture the dose of vaccine was in all instances 0.001 cc. of an 18 hour broth culture diluted to 1 cc. with normal salt solution. This amount of culture contained approximately 400,000 pneumococci. In vaccinating with the avirulent strain a dose of 1 to 2 cc. of undiluted broth culture was employed.

Reactions.—The local reaction to living pneumococcus vaccine was very mild in the monkeys which were studied, consisting of merely a slight induration, free from redness and tenderness. The constitutional reaction depended, on the one hand, upon the dose and virulence of the culture, and, on the other hand, upon the individual resistance of the monkey. The latter is a factor of considerable variation. As the experiments will show, the constitutional reactions varied widely in severity, from no reaction whatever to a fatal pneumococcus septicemia.

The resistance of the vaccinated monkeys to experimental pneumonia was tested 2 or 3 weeks after vaccination by the intratracheal injection of virulent pneumococci, as described in Paper I.[6]

[5] Strong, R. P., *J. Med. Research*, 1908, xiii, 325.

[6] Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, xxxi, 403.

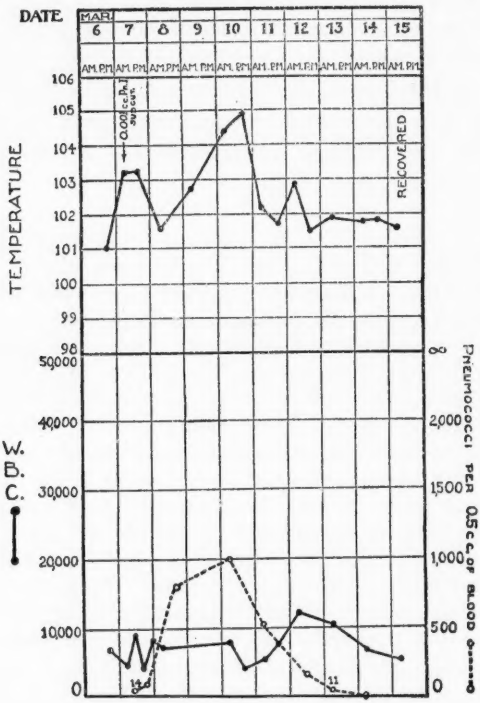
Vaccination with a Living Virulent Culture of Pneumococcus.

The strain of pneumococcus used as a vaccine in this group of experiments was a highly virulent Pneumococcus Type I which killed a mouse regularly in doses of 0.0000001 cc. of broth culture. The same strain was used for the intratracheal injections, and when injected into the trachea of monkeys in doses of 0.000001 cc. of broth culture would produce in practically every instance a classic lobar pneumonia.

Reaction of Monkeys to Subcutaneous Injection of Living Virulent Pneumococci.—The first monkey vaccinated with a living virulent pneumococcus was studied in considerable detail. The protocol therefore will be reported in full.

Experiment 1.—Monkey 7. *Cebus capucinus*, female; weight, 1,430 gm. Mar. 7, 1919, 10.30 a.m. Well and active. Subcutaneous injection of 0.001 cc. of 18 hour broth culture of Pneumococcus Type I. 1.30 p.m. Well and active. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 14 colonies of Pneumococcus Type I. 4.30 p.m. Appears well and active. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 84 colonies of Pneumococcus Type I. 7.30 p.m. Appears well and active. Mar. 8, 10.30 a.m. Appears well and active. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 800 colonies (approximately) of Pneumococcus Type I. Mar. 9. Appears well and active. Mar. 10, 9 a.m. Monkey quiet at times, restless at times; evidently becoming sick. 10.30 a.m. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 1,000 colonies (approximately) of Pneumococcus Type I. 4 p.m. Appears sick; offers little resistance to handling; respirations not increased. Blood culture: one loop on surface of blood agar plate, 18 colonies of Pneumococcus Type I. Mar. 11, 10.45 a.m. Appears better; more active. Blood culture: 0.1 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar, 510 colonies of Pneumococcus Type I. 4.30 p.m. X-ray of chest. There is no evidence of consolidation in the chest. Mar. 12, 10 a.m. Appears well and active. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 151 colonies of Pneumococcus Type I. Mar. 13, 10.30 a.m. Appears well and active. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 11 colonies of Pneumococcus Type I. Mar. 14, 11 a.m. Well and active. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, no growth. Mar. 15. Well and active. Mar. 27. Well and active. Monkey bled (10 cc.). Serum tests with Pneumococcus Type I: Agglutinins, 0. Protection, 0.0001 cc., survived; 0.00001 cc., survived; 0.000001 cc., survived 4½ days. Control, 0.00001 cc., died in 48 hours; 0.000001 cc., died in 48 hours.

Text-fig. 1 shows the temperature, leucocyte, and blood culture curves following vaccination. There was a sharp rise of temperature in response to the injection, but the leucocytes were unaffected. The most striking feature of the chart is the blood culture curve. The pneumococci invaded the blood stream within 3 hours after vaccination,


DATE
MAR.
6 7 8 9 10 11 12 13 14 15
AM. PM. AM. PM. AM. PM. AM. PM. AM. PM. AM. PM. AM. PM. AM. PM. AM. PM. AM. PM.
TEMPERATURE
106 105 104 103 102 101 100 99 98
0.001 c.c. [illegible] broth culture
RECOVERED
W. B. C.
50,000 40,000 30,000 20,000 10,000 0
PNEUMOCOCCI PER 0.5 c.c. OF BLOOD
8 2,000 1,500 1,000 500 0
14
11

TEXT-FIG. 1. Monkey 7. Reaction following subcutaneous inoculation of 0.001 cc. of broth culture of living virulent Pneumococcus Type I.

and by the end of 24 hours there were nearly 1,000 pneumococci per 0.5 cc. of blood. In spite of this septicemia, however, the monkey did not appear seriously ill at any time. The blood cleared up rapidly, the temperature dropped, and 5 days after vaccination the monkey appeared well and active. It is surprising that a heavy septicemia of this kind could have been associated with such mild clinical symptoms.

It is also noteworthy that 20 days after vaccination the serum of this monkey protected mice against 1,000 times the minimal lethal dose of Pneumococcus Type I.

Experiment 2.—Apr. 15, 1919. Three *Macacus syrichtus* monkeys (Nos. 46, 47, and 48) were inoculated with a living virulent Pneumococcus Type I, each receiving the same dose that Monkey 7 received—0.001 cc. of broth culture subcutaneously. Condensed protocols are shown in Table I and Text-fig. 2.

TABLE I.

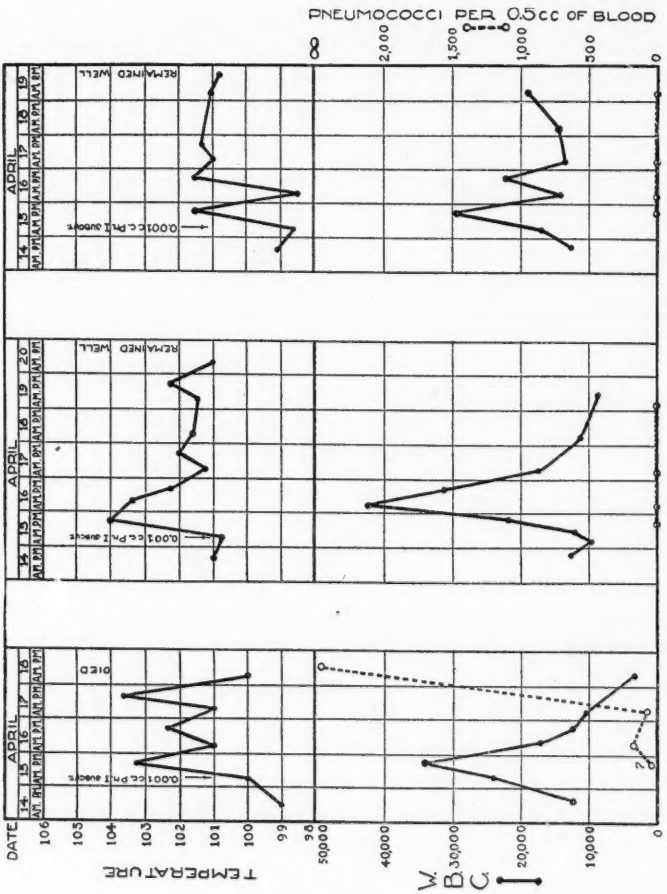
Effect of Subcutaneous Injection of Living Culture of Virulent Pneumococcus Type I.

Monkey No.	Weight.	Apr. 15. Broth culture of Pn. I subcutaneously.	Apr. 29. Serum tests with Pn. I.			Result.	Autopsy.	Autopsy cultures. Heart's blood.
			Agglutinins.	Protection.	Control.			
	gm.	*cc.*						
46	2,550	0.001				D. 4 days.	Lungs normal.	Pn. I
47	2,710	0.001	1:1++	0.00001 cc. S.* 0.000001 cc. S. 0.0000001 cc. S.	0.00001 cc. D. 24 hrs. 0.000001 cc. D. 36 hrs. 0.0000001 cc. D. 36 hrs.	Remained well.		
48	3,590	0.001	0	0		Remained well.		

* S. indicates survived; D., died.

It will be observed that each of these three monkeys reacted differently to the vaccine. The smallest one (Monkey 46) showed a severe constitutional reaction with heavy septicemia, and died on the 4th day. Monkey 47 reacted with a sharp but temporary rise in temperature and leucocytes, but the blood remained sterile and the monkey at no time appeared ill. The largest monkey (No. 48) showed no reaction of any kind except a slight leucocytosis.

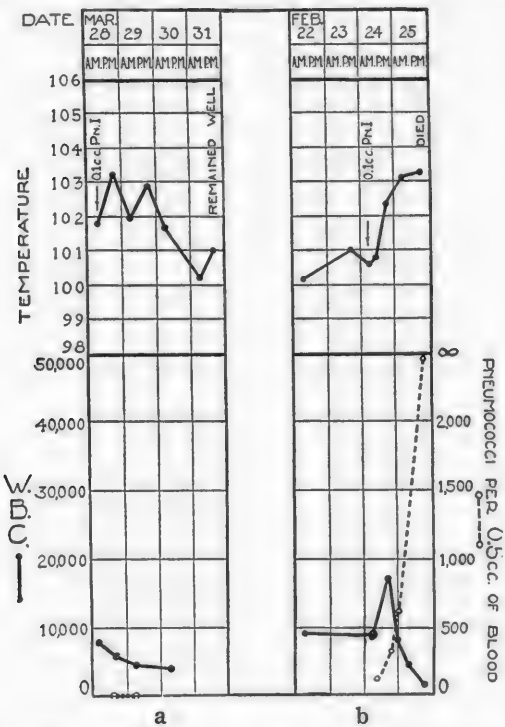
2 weeks after vaccination the two surviving monkeys were bled and their sera tested for agglutinins and protective bodies against Pneumococcus Type I. Monkey 47 showed both agglutinins and protective substances. Its serum protected mice against 100 times the minimal lethal dose of Pneumococcus Type I. Monkey 48, on the other hand,



TEXT-FIG. 2, *a*, *b*, and *c*. Reactions following subcutaneous inoculation of living virulent Pneumococcus Type I. (*a*) Monkey 46, (*b*) Monkey 47, (*c*) Monkey 48. Each of these animals received 0.001 cc. of living culture subcutaneously.

exhibited neither agglutinins nor protective bodies. These reactions indicate what a large factor individual variation may be in experiments of this kind.

Active Immunity Following Subcutaneous Injection of Living Virulent Pneumococci.—The immediate effect of subcutaneous injections of



TEXT-FIG. 3, *a* and *b*. Active immunity against Pneumococcus Type I following vaccination with living virulent Pneumococcus Type I. (*a*) Monkey 7; received 0.001 cc. of living culture subcutaneously. (*b*) Monkey 3; control.

living virulent pneumococci having been studied, the next step was to determine the degree of immunity conferred by this method of vaccination.

The object of the two following experiments was to test the vaccinated monkeys for active immunity against Pneumococcus Type I.

TABLE II.

Active Immunity Following Subcutaneous Injection of a Living Culture of Virulent Pneumococcus Type I.

Monkey No.	Weight.	Mar. 7. Broth culture of Pn. I subcutaneously.	Mar. 11. X-ray.	Mar. 27. Serum tests with Pn. I.			Mar. 28. Broth culture of Pn. I intratracheally.	Result.	Autopsy.	Autopsy cultures.	
				Agglutinins.	Protection.	Control.				Lung.	Heart's blood.
	gm.	*cc.*					*cc.*				
7	1,430	0.001	Negative.	0	0.0001 cc. S. 0.00001 cc. S. 0.000001 cc. S. 4½ days.	0.00001 cc. D. 48 hrs. 0.000001 cc. D. 48 hrs.	0.1	Remained well.			
3	1,595	0	" (Feb. 19).				0.1 (Feb. 24).	D. 2 days.	Lobar pneumonia; red stage.	Pn. I	Pn. I

The identical strain of Pneumococcus Type I was used for the intratracheal injections that had been used for the subcutaneous vaccinations.

Experiment 3.—Mar. 28, 1917. Monkey 7, 21 days after vaccination with living virulent Pneumococcus Type I, was injected intratracheally with 0.1 cc. of an 18 hour Pneumococcus Type I broth culture. No monkey of this species was available as an actual control at the time. The record of Monkey 3, however, is introduced to indicate what had been the invariable result when comparable doses of Pneumococcus Type I were injected intratracheally in normal unvaccinated *capucinus* monkeys. The temperature, leucocyte, and blood culture curves are charted in Text-fig. 3. The results are shown in Table II.

The vaccinated monkey (No. 7), though it received 100,000 times the minimal infecting dose, remained perfectly well, while Monkey 3 died with incipient lobar pneumonia and an overwhelming pneumococcus septicemia on the day following injection. Even the leucocyte count in Monkey 7 remained unchanged.

In other words, a very small quantity of living virulent pneumococcus culture (0.001 cc.) injected subcutaneously was sufficient to confer on the monkey a high degree of active immunity against the homologous strain.

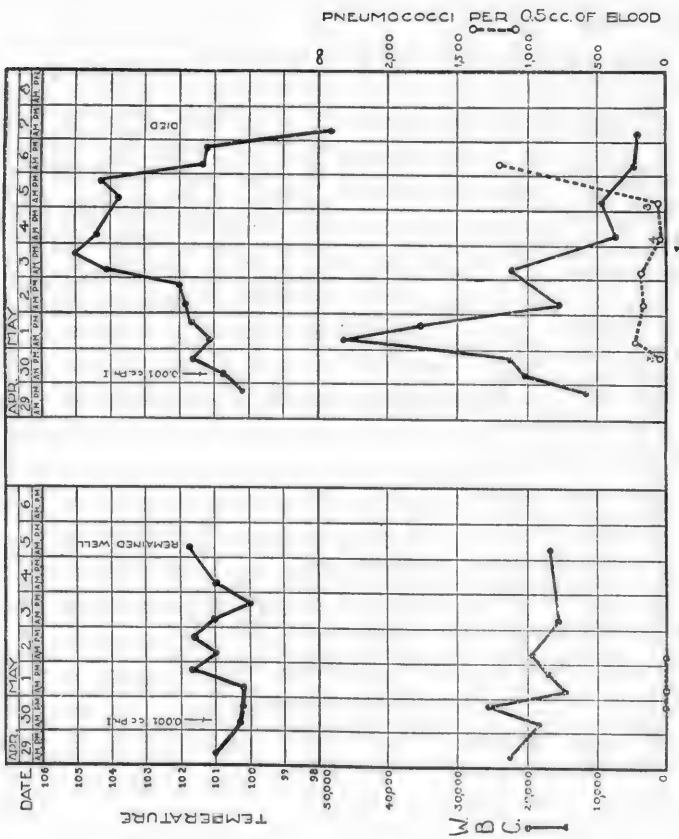
One of the *Macacus* monkeys (No. 48) which had been vaccinated with living virulent pneumococci (0.001 cc.) was next tested.[7]

Experiment 4.—Apr. 30, 1919. Monkey 48, vaccinated on Apr. 15 with 0.001 cc. of living Pneumococcus Type I, and Monkey 83, control, were each injected intratracheally with 0.001 cc. of Pneumococcus Type I broth culture.

The protocols (Table III and Text-fig. 4) show that the vaccinated monkey remained active and well. The control developed a typical lobar pneumonia and died on the 8th day with the usual pneumococcus septicemia. As in the previous experiment, the vaccinated monkey showed no rise in temperature and did not even react with a leucocytosis.

There was, however, a difference between the vaccinated monkey in Experiment 3 and the one in Experiment 4. The former developed a considerable amount of protective substance in the blood following vaccination; the latter showed none. Yet both were immune to Pneumococcus Type I pneumonia.

[7] Monkey 47 will be considered later.



TEXT-FIG. 4, *a* and *b*. Active immunity against Pneumococcus Type I following vaccination with living virulent Pneumococcus Type I. (*a*) Monkey 48; received 0.001 cc. of living culture subcutaneously. (*b*) Monkey 83; control.

TABLE III.

Active Immunity Following Subcutaneous Injection of a Living Culture of Virulent Pneumococcus Type I.

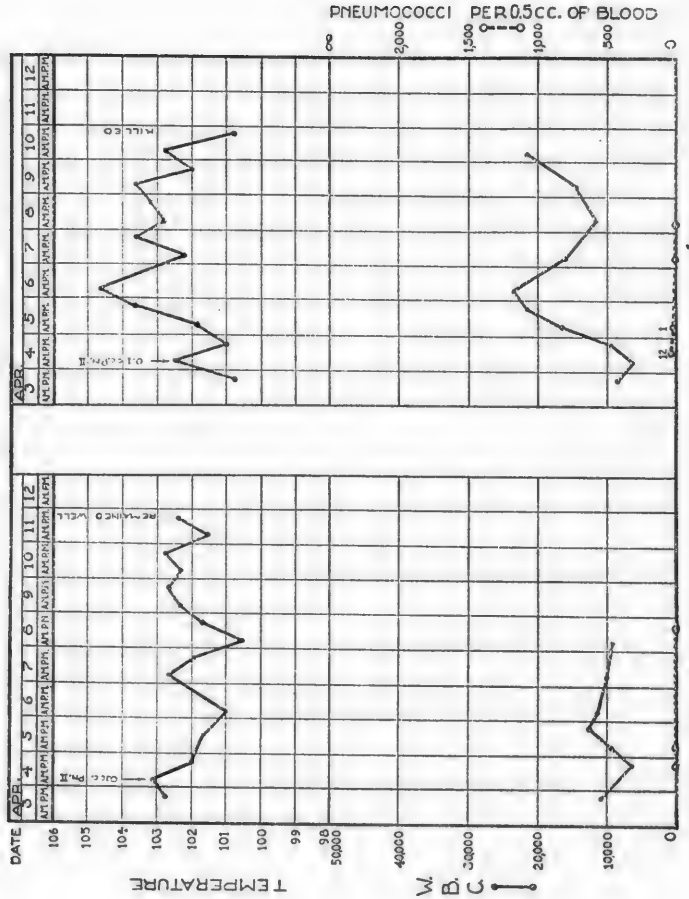
Monkey No.	Weight.	Apr. 15. Broth culture of Pn. I subcutaneously.	Apr. 30. Broth culture of Pn. I intratracheally.	May 2. X-ray.	Result.	Autopsy.	Autopsy cultures.	
							Lung.	Heart's blood.
	gm.	*cc.*	*cc.*					
48	3,590	0.001	0.001	Negative.	No signs of pneumonia. Remained well.			
83	2,470	0	0.001		Clinical pneumonia. D. 8th day.	Lobar pneumonia, R. U., R. M., R. L.*	Pn. I	Pn. I

*R. L., R. M., R. U., etc., indicate lobes of the lung. The cardiac lobe is included as part of the right lower lobe.

Cross-Immunity against Pneumococcus Type II Following Vaccination with Living Virulent Pneumococcus Type I.—The experiments just reported afford ample proof that vaccination with a living virulent Pneumococcus Type I stimulates a high degree of active immunity against the homologous type of pneumococcus. The next step was to determine whether vaccination with living Pneumococcus Type I afforded any cross-immunity against the other types of pneumonia. 1 week, therefore, after the test against Pneumococcus Type I, Monkey 7 was tested against a virulent strain of Pneumococcus Type II.

Experiment 5.—Apr. 4, 1919. Monkey 7, vaccinated against Pneumococcus Type I, and Monkey 25, control, were injected intratracheally with 0.1 cc. of broth culture of Pneumococcus Type II (Table IV, Text-fig. 5). Monkey 7 remained perfectly well, with no rise in temperature or leucocytes. Monkey 25, the control, developed lobar pneumonia with leucocytosis and positive blood culture. The control recovered by crisis on the 7th day.

According to this experiment vaccination with a living virulent Pneumococcus Type I had conferred not only immunity against the homologous type but a cross-immunity against Pneumococcus Type II as well. Furthermore, this immunity existed in spite of the absence of protective bodies against Pneumococcus Type II in the serum of the monkey.



TEXT-FIG. 5, *a* and *b*. Cross-immunity against Pneumococcus Type II following vaccination with living virulent Pneumococcus Type I. (*a*) Monkey 7; received 0.001 cc. of Pneumococcus Type I living culture subcutaneously. (*b*) Monkey 25; control.

TABLE IV.

Production of Cross-Immunity with a Living Culture of Virulent Pneumococcus Type I.

Monkey No.	Weight.	Mar. 31. Serum tests with Pn. II.		Apr. 4. Broth culture of Pn. II intratracheally.	Apr. 7. X-ray.	Result.	Autopsy.	Autopsy cultures.	
		Agglutinins.	Protection.					Lung.	Heart's blood.
	gm.			*cc.*					
7	1,430	0	0	0.1	Negative.	Remained well.			
25	740			0.1	Shadow, R. U.	Clinical pneumonia. Crisis on 7th day. Killed.	Lobar pneumonia, R. U.; gray stage.	Sterile.	Sterile.

Experiment 6.—June 24, 1919. 8 weeks after the test against Pneumococcus Type I, Monkey 48, vaccinated against Pneumococcus Type I, and Monkey 91, control, were injected intratracheally with 0.1 cc. of broth culture of Pneumococcus Type II (Table V, Text-fig. 6).

Both monkeys developed Type II pneumonia and both recovered. In the vaccinated monkey, however, the disease ran a mild short course, and the blood was sterile throughout practically the entire course of the disease. The control monkey ran a long course (crisis on the 18th day) and showed a fairly heavy pneumococcus septicemia.

In the case, then, of Monkey 48 successful vaccination against Type I pneumonia did not give sufficient cross-immunity to protect against Pneumococcus Type II pneumonia, though it did apparently moderate the severity of the disease. The inconsistency between the results obtained in Experiments 5 and 6 may be explained in several ways. In Experiment 5, the test for cross-immunity against Pneumococcus Type II was made 1 week after the test against Pneumococcus Type I; in Experiment 6 the test for cross-immunity was carried out 8 weeks after testing for homologous immunity. The duration of pneumococcus immunity is not known, but a high degree of resistance lasts probably not more than a few months. Another ex-

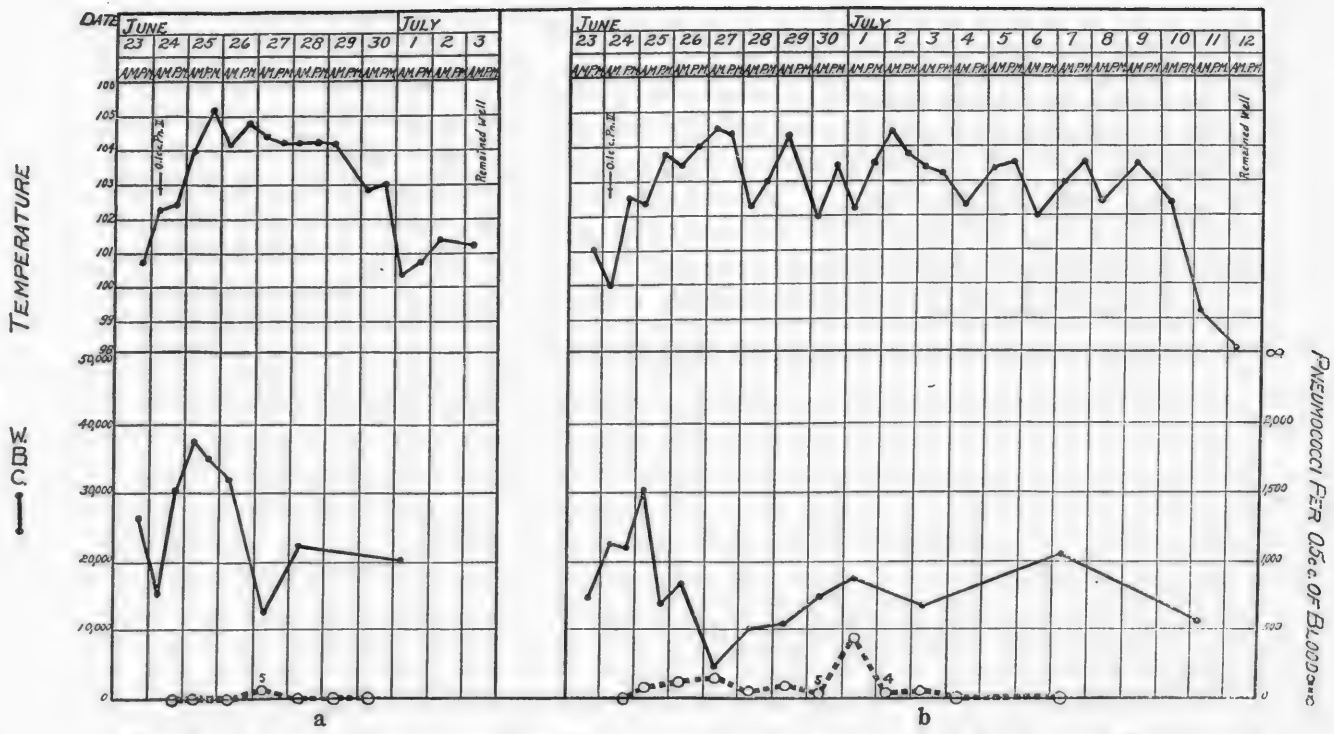
planation may be found in the much severer reaction exhibited by Monkey 7 following vaccination, with a consequent development of a higher grade of immunity. Still another and perhaps more rational explanation would interpret this difference in cross-immunity to individual variation in the monkeys.

TABLE V.

Experimental Pneumococcus Type II Pneumonia Following Vaccination with a Living Culture of Virulent Pneumococcus Type I.

Monkey No.	Weight.	Apr. 15. Broth culture of Pn. I subcutaneously.	Apr. 30.	June 23. Serum tests with Pn. II.			June 24. Broth culture of Pn. II intratracheally.	Result.
				Agglutinins.	Protection.	Control.		
	gm.	*cc.*					*cc.*	
48	3,590	0.001	Resisted infection with Pn. I intratracheally.	0	0.000001 cc. S. 0.00001 cc. S. 0.0001 cc. D. 48 hrs. 0.001 cc. D. 48 hrs.	0.000001 cc. D. 48 hrs. 0.00001 cc. D. 20 hrs. 0.0001 cc. D. 18 hrs.	0.1	Clinical pneumonia. Recovery by crisis on 8th day.
91 (control).	4,002	0					0.1	Clinical pneumonia. Recovery on 18th day.

There is one more interesting feature to this experiment. It will be recalled that Monkey 48, though immune to Pneumococcus Type I pneumonia showed no protective substance in the blood. When, however, 8 weeks later a second protection test was carried out on the serum of this monkey, this time against Pneumococcus Type II, a definite though slight amount of protection was demonstrated. We offer no explanation of this phenomenon.



TEXT-FIG. 6, *a* and *b*. Pneumococcus Type II pneumonia following vaccination with living virulent Pneumococcus Type I. (*a*) Monkey 48; received 0.001 cc. of living culture of Pneumococcus Type I subcutaneously. (*b*) Monkey 91; control.

Cross-Immunity against Pneumococcus Type III Following Vaccination with Living Virulent Pneumococcus Type I.—The last two experiments (Nos. 5 and 6) gave some evidence of a cross-immunity against Pneumococcus Type II following vaccination with living virulent Type I pneumococci. It was decided, therefore, to determine whether a similar cross-protection existed against Pneumococcus Type III.

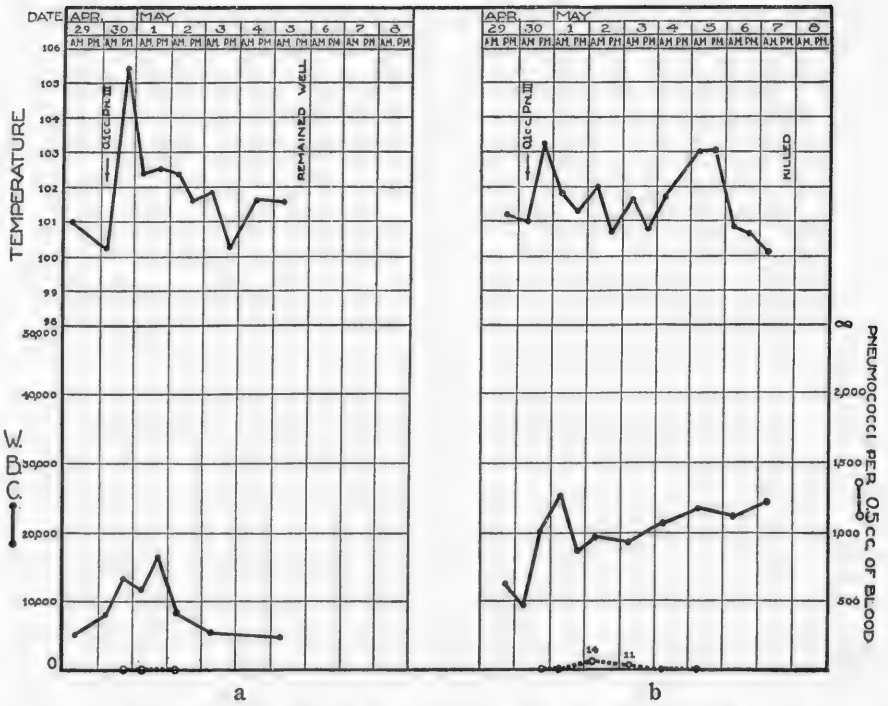
TABLE VI.

Production of Cross-Immunity with a Living Culture of Virulent Pneumococcus Type I.

Monkey No.	Weight.	Apr. 29. Serum tests with Pn. III.		Apr. 30. Broth culture of Pn. III intratracheally.	May 3. X-ray.	Result.	Autopsy.	Autopsy cultures	
		Agglutinins.	Protection.					Lung.	Heart's blood.
	gm.			*cc.*					
7	1,430	0	0	0.1	Negative.	Temporary febrile reaction; no pneumonia.			
82	732			0.1	Shadow, R. U.	Clinical pneumonia. Crisis on 7th day. Killed.	Interstitial pneumonia, R. U	Sterile.	Sterile.

Experiment 7.—Apr. 30, 1919. About 4 weeks after the test against Pneumococcus Type II, Monkey 7, vaccinated with living culture of Pneumococcus Type I, and Monkey 82, control, were injected intratracheally with 0.1 cc. of broth culture of a virulent Pneumococcus Type III. The results are shown in Table VI and Text-fig. 7.

The vaccinated monkey reacted to the injection of Pneumococcus Type III with a sharp but temporary rise of temperature, and a slight increase in leucocytes. The blood culture remained sterile. The monkey appeared sick during the afternoon of the day on which it was injected, but the next morning it was lively and well and remained so. The *x*-ray was negative, and if any pneumonia developed it must



TEXT-FIG. 7, *a* and *b*. Partial cross-immunity against Pneumococcus Type III following vaccination with living virulent Pneumococcus Type I. (*a*) Monkey 7; received 0.001 cc. of living culture of Pneumococcus Type I subcutaneously. (*b*) Monkey 82; control.

have been an exceedingly small patch. On the other hand, the control monkey developed a definite but rather mild attack of Type III pneumonia, with moderate leucocytosis and positive blood culture. The control recovered by crisis on the 7th day and autopsy showed a resolving interstitial pneumonia.

Vaccination with a living virulent Pneumococcus Type I apparently gave Monkey 7 a certain amount of cross-immunity against Pneumococcus Type III as well as against Type II. As with Pneumococcus Type II, the monkey possessed no demonstrable protective bodies in the blood against Pneumococcus Type III.

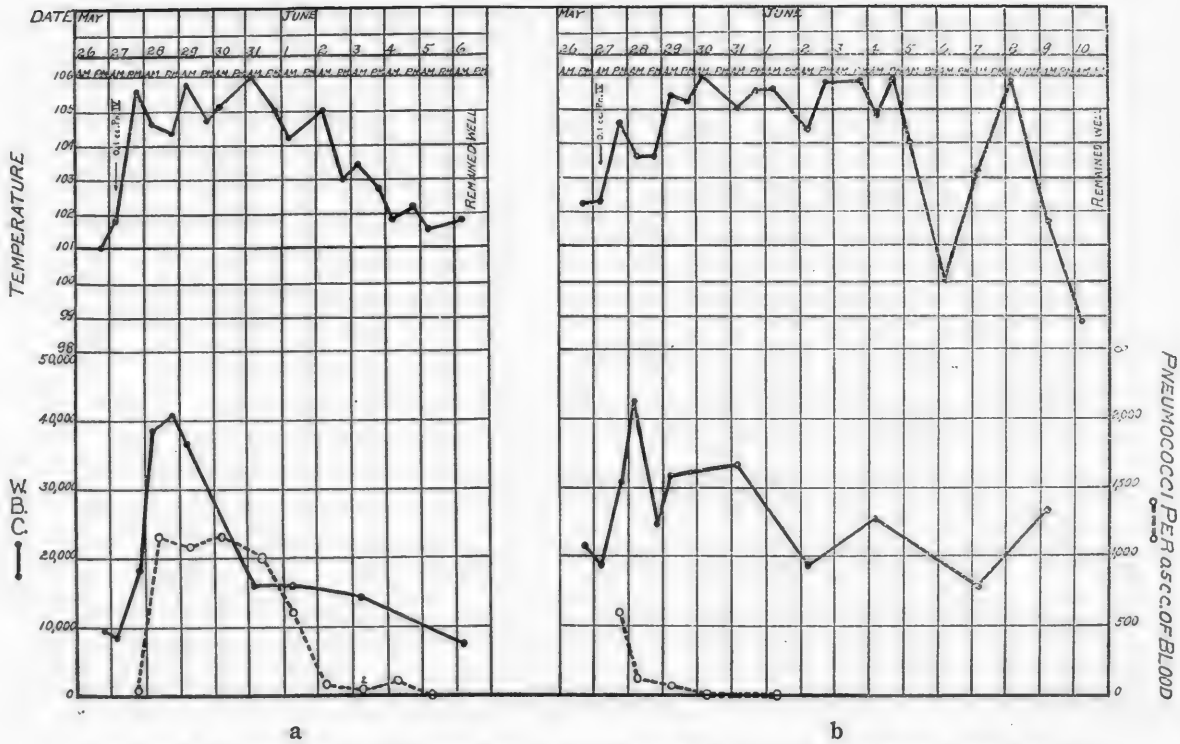
Test for Cross-Immunity against Pneumococcus Type IV Following Vaccination with Living Virulent Pneumococcus Type I.—One of the

TABLE VII.

Experimental Pneumococcus Type IV Pneumonia Following Vaccination with a Living Culture of Virulent Pneumococcus Type I.

Monkey No.	Weight.	May 27. Broth culture of Pn. IV intratracheally.	June 5. X-ray.	Result.	Autopsy.	Autopsy cultures.	
						Lung.	Heart's blood.
	gm.	*cc.*					
7	1,430	0.1	Negative.	Clinical pneumonia. Recovery by lysis on 8th day. Killed on 14th day.	Resolving lobar pneumonia, R. L., L. L.	No growth.	No growth.
111	2,219	0.1	Shadow, R. L.	Clinical pneumonia. Recovery on 14th day.			

vaccinated monkeys (No. 7) had resisted Pneumococcus Type I, Type II, and probably Type III pneumonia. It remained, therefore, only to test it against Pneumococcus Type IV. In this test a strain of Type IV was employed that had been recently isolated from a case of spontaneous Pneumococcus Type IV pneumonia in one of the stock monkeys (No. 97).



TEXT-FIG. 8, *a* and *b*. Pneumococcus Type IV pneumonia following vaccination with living virulent Pneumococcus Type I. (*a*) Monkey 7; received 0.001 cc. of living culture of Pneumococcus Type I subcutaneously. (*b*) Monkey 111; control.

Experiment 8.—May 27, 1919. 4 weeks after being tested for immunity against Pneumococcus Type III, Monkey 7, vaccinated, and Monkey 111, control, were injected intratracheally with 0.1 cc. of broth culture of Pneumococcus Type IV (Strain M 97). Table VII and Text-fig. 8 show the results of the experiment.

Both monkeys developed Type IV pneumonia with high temperature, leucocytosis, pneumococcus septicemia, and final recovery; but the vaccinated monkey was well on the 8th day, while the control did not recover until the 14th day.

Apparently, there was not enough cross-immunity to protect the vaccinated animal against pneumonia, though there was enough, as with Monkey 48 in Experiment 6, to moderate and shorten somewhat the course of the disease. Just why Monkey 7 resisted the three fixed types of pneumococcus and then became infected with a Pneumococcus Type IV it is hard to say. Nearly 3 months had elapsed from the day of vaccination to the day when the monkey was injected with Pneumococcus Type IV, and it may be that by that time the immunity curve was on the downward way. Moreover, the pneumococcus used had recently been isolated from another infected monkey, and was therefore adapted, in a sense, to the species. The fixed strains of pneumococcus used had never been passed through monkeys.

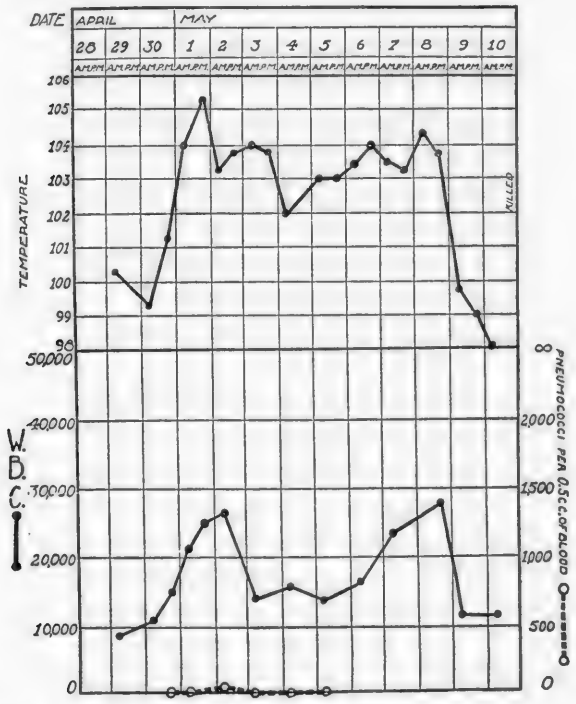
Spontaneous Pneumococcus Type IV Pneumonia Following Vaccination with a Living Virulent Pneumococcus Type I.—The following case corroborates the evidence obtained in the last experiment; namely, that there is not much cross-protection against Pneumococcus Type IV pneumonia in monkeys following vaccination with a living virulent culture of Pneumococcus Type I.

Experiment 9.—Monkey 47. *Macacus syrichtus*, male; weight 2,170 gm. Apr. 15, 1919, 10 a.m. Vaccinated with 0.001 cc. of 18 hour broth culture of Pneumococcus Type I (see Experiment 2). Apr. 30. Well and active. May 1. Looks sick; rapid labored respiration. May 2. Blood culture, Pneumococcus Type IV. May 3. Bronchial breathing in left axilla. May 4. Dyspnea persists; cough. May 5. Condition the same. May 9. Crisis and recovery. May 10. Killed.

Autopsy.—Resolving lobar pneumonia, left middle and lower lobes and right middle lobe; acute fibrinous pleuritis, left.

Text-fig. 9 shows the temperature, leucocyte, and blood culture curves of this case of spontaneous pneumonia. The disease ran a typical course, very similar to that seen in the experimental pneumo-

nia of monkeys. The blood, however, remained practically sterile (only one positive blood culture), and this fact suggests that here as in Monkey 7, Pneumococcus Type I vaccination afforded enough cross-immunity against Pneumococcus Type IV to moderate the severity of the pneumonia caused by the latter organism.

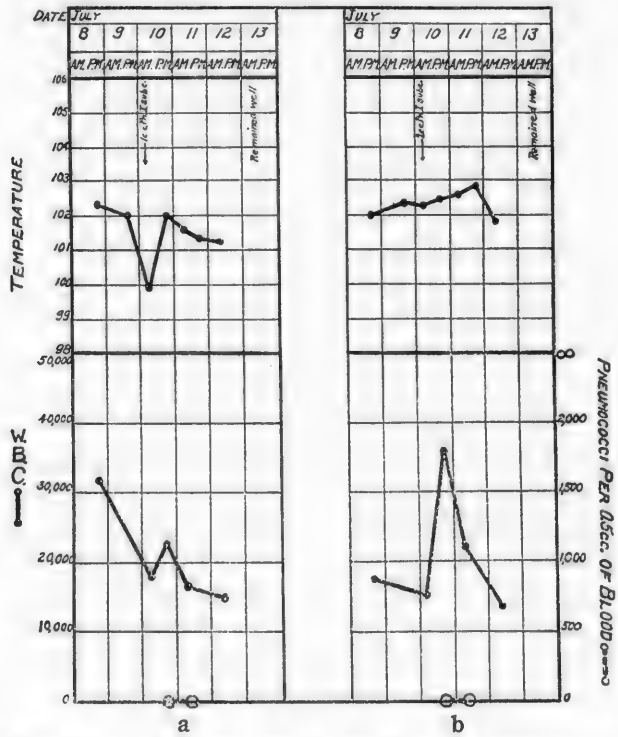


TEXT-FIG. 9. Monkey 47. Spontaneous Pneumococcus Type IV pneumonia in a monkey vaccinated with living virulent Pneumococcus Type I. Received 0.001 cc. of living culture of virulent Pneumococcus Type I subcutaneously.

Vaccination with a Living Avirulent Culture of Pneumococcus.

Up to this point all the experiments reported have shown the results obtained by vaccination with a living virulent pneumococcus. The remainder of the vaccination experiments were carried out with

a living avirulent pneumococcus, in order to determine how important a factor virulence was, on the one hand, and, on the other, how much depended upon the use of living or killed cultures.



TEXT-FIG. 10, *a* and *b*. Reactions following the subcutaneous inoculation of living avirulent Pneumococcus Type I. (*a*) Monkey 117; received 1 cc. of living culture subcutaneously. (*b*) Monkey 118; received 2 cc. of living culture subcutaneously.

The culture used for vaccination in the following experiments was an old stock Pneumococcus Type I which was avirulent for mice in doses of 1 cc. of broth culture. This strain was used for the preparation of the vaccine in the study of vaccination with killed cultures.[8]

[8] Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, xxxi, 499.

It was assumed that in view of the lack of virulence a much larger dose of vaccine would be necessary in order to obtain results; consequently, a dose of 1 to 2 cc. of broth culture was employed. The injections were given subcutaneously in the abdominal wall as in the previous experiments.

Experiment 10.—July 10, 1919. Two *Macacus syrichtus* monkeys (Nos. 117 and 118) were injected subcutaneously with an 18 hour broth culture of living avirulent Pneumococcus Type I. Monkey 117 received 1 cc. of culture (about 300 million pneumococci). Monkey 118 received 2 cc. (about 600 millon pneumococci). The reactions are shown in Text-fig. 10.

Monkey 117 developed a small area of induration at the site of inoculation but had no constitutional reaction whatever. Monkey 118 also showed a slight local reaction, and in addition a moderate rise in leucocytes. The blood remained sterile in both monkeys.

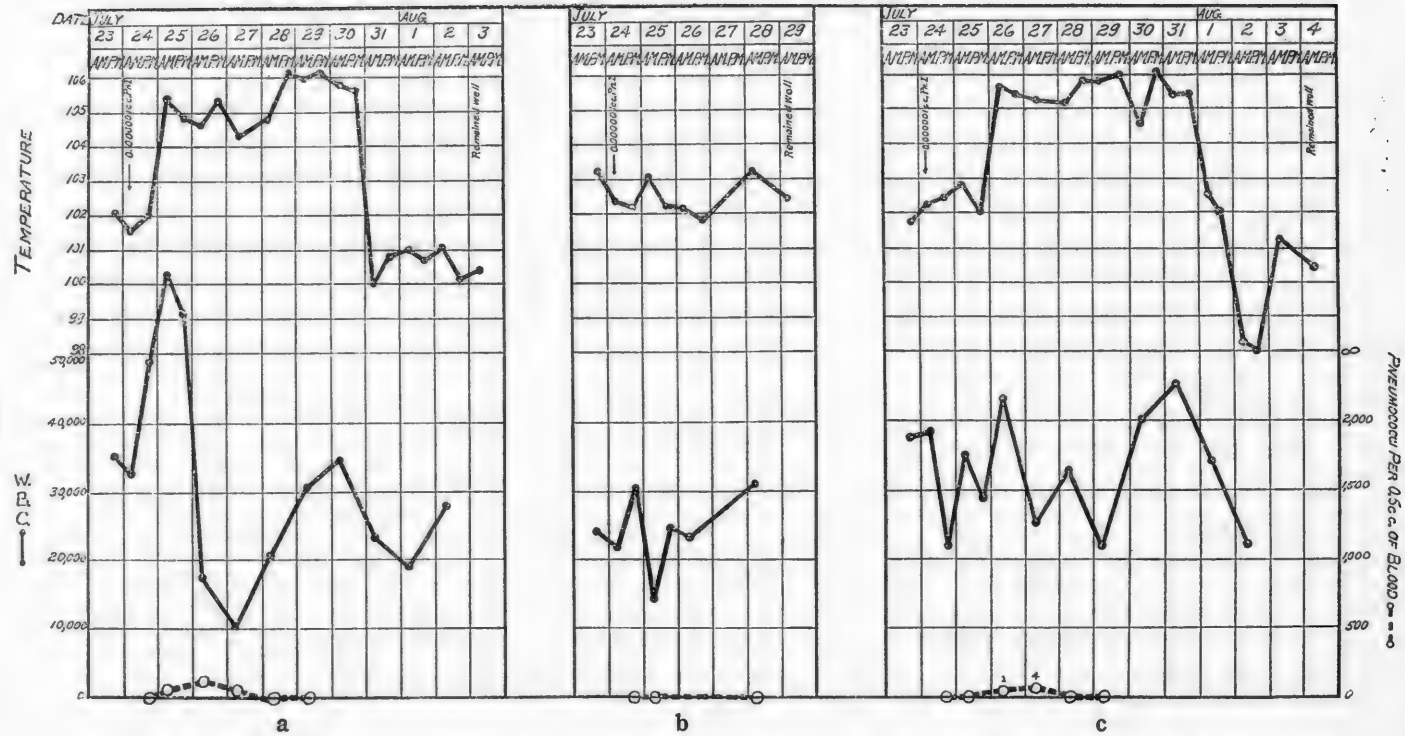
TABLE VIII.

Active Immunity Following Subcutaneous Injection of a Living Culture of Avirulent Pneumococcus Type I.

Monkey No.	Weight.	July 10. Broth culture of Pn. I subcutaneously.	Reaction.	July 24. Serum tests with Pn. I.		July 24. Broth culture of Pn. I intratracheally.	Result.
				Agglutinins.	Protection.		
	gm.	*cc.*				*cc.*	
117	2,565	1	Slight; local.	0	0	0.000001	Clinical pneumonia. Recovery by crisis on 8th day.
118	2,915	2	Slight; local.	0	0	0.000001	Remained well.
127 (control).	2,700	0				0.000001	Clinical pneumonia. Recovery by crisis on 9th day.

This experiment indicated that living avirulent pneumococci could be administered subcutaneously in fairly large doses to monkeys without producing any serious local or general reaction.

The following experiment was carried out for the purpose of testing the amount of active immunity produced by vaccination with living avirulent pneumococci.



TEXT-FIG. 11, *a*, *b*, and *c*. Active immunity (Monkey 118) against Pneumococcus Type I following vaccination with 2 cc. of living avirulent Pneumococcus Type I; 1 cc. of the same culture failed to protect Monkey 117. (*a*) Monkey 117; received 1 cc. of living avirulent culture subcutaneously. (*b*) Monkey 118; received 2 cc. of living avirulent culture subcutaneously. (*c*) Monkey 127; control.

Experiment 11.—July 24, 1919. Monkeys 117 and 118, both vaccinated on July 10 with living avirulent pneumococci, and Monkey 127, control, were injected intratracheally with 0.000001 cc. of broth culture of Pneumococcus Type I (Table VIII). Monkey 117, which received the small dose (1 cc.) of living vaccine, developed a mild pneumonia and recovered by crisis on the 8th day of the disease. Monkey 118, which received the large dose (2 cc.) of living vaccine, remained perfectly well. The control (Monkey 127) developed pneumonia and ran a typical course with crisis on the 9th day.

Text-fig. 11 shows the temperature, leucocyte, and blood culture curves. Monkey 118 presented no reaction of any kind. Monkey 117 ran a typical lobar pneumonia, with positive blood culture, high leucocytosis, and crisis on the 8th day. Monkey 127 also ran a typical course with crisis on the 9th day.

Living avirulent pneumococci, when injected subcutaneously, appear to excite an immunity equal in degree to that produced by living virulent pneumococci, if a large enough dose is administered. In this experiment 1 cc. of broth culture of living avirulent pneumococci did not confer a sufficiently high degree of immunity to protect the monkey from pneumonia. When 2 cc., however, of the same culture were administered to another monkey, the protection was satisfactory. In other words, the dose is a factor to be considered in vaccinating with living avirulent pneumococci.

DISCUSSION.

The inferences to be drawn from the experiments reported in this study are plain. A high degree of immunity against pneumococcus pneumonia can be induced by the subcutaneous injection of living virulent pneumococci, but the method is too dangerous for any sort of practical application. Vaccination with attenuated living pneumococci could probably be practised with impunity, but the problem of transporting and keeping alive large quantities of pneumococci in the field would be difficult to solve. The fact that a higher degree of immunity is produced by living pneumococci than by dead pneumococci is not surprising in the light of previous observations on vaccination with other bacteria. The degree of cross-immunity, however, which sometimes followed vaccination with living Pneumococcus Type I was surprising and confirms the fact already established that the various types of pneumococci are closely related biologically.

This study had suggested a number of theoretical problems. In the first place, why do living pneumococci confer a more efficient immunity than dead pneumococci? Virulence appears to play some part, but even with avirulent strains good protection can be secured with sufficiently large doses. These animals vaccinated with living cultures are, of course, in a sense infected, and we are accustomed to think of infection as bestowing a more efficacious immunity than mere vaccination with killed cultures.

The question also arises as to the significance of agglutinins and the so called protective bodies in an animal's blood. It has been shown in the experiments reported that the serum of a monkey may be entirely free from these substances, and yet the animal may possess a high grade of immunity against pneumonia. On the other hand, it was pointed out in the study of pneumococcus saline vaccines[1] that the serum of a vaccinated monkey might protect mice against 100 or even 1,000 minimal lethal doses of pneumococci, and still that monkey be susceptible to experimental pneumococcus pneumonia. These facts complicate the whole question of resistance to pneumococcus infection and revive the old problem of humoral *versus* cellular immunity.

These studies also emphasize the fact that "immunity" is a relative term and under any circumstances dependent on a number of factors. Every immunologist is familiar with the great differences which animals manifest in their capacity to produce antibodies. Even with the most ideal methods of vaccination there will always be certain animals that respond poorly to vaccine, and these animals will not possess the same amount of immunity that others will have.

Furthermore, the degree of immunity against a certain microorganism depends in large measure on the virulence of the strain used in testing it. For example, in the experiments on cross-immunity, Monkey 7 resisted infection with Pneumococcus Type III which was not very virulent for monkeys, but could not resist Pneumococcus Type IV which, in this particular instance, happened to be virulent for monkeys. In view of this fact the question arises whether in the experiments with Pneumococcus Type I lipovaccine and saline vaccine immunity would not have been demonstrated if a less virulent strain of Pneumococcus Type I had been used for testing the efficacy of the vaccine.

All that has been said regarding virulence applies with equal force to the size of the infecting dose. Even in well immunized monkeys experimental pneumonia could probably have been induced if a sufficiently large dose of culture had been injected into the trachea. This would have been a most artificial procedure, but the argument holds in spite of that fact. The animal possesses a definite sum of resistance, and when that sum is spent it becomes at once susceptible to infection.

CONCLUSIONS.

1. The subcutaneous injection of small doses of living virulent Pneumococcus Type I stimulates in monkeys a degree of active immunity sufficient to protect them against experimental pneumococcus pneumonia of homologous type.

2. The subcutaneous injection of living avirulent Pneumococcus Type I, if administered in a sufficiently large dose, likewise renders the monkey immune to a subsequent pneumonia of homologous type.

3. Vaccination of monkeys with small doses of living virulent pneumococci may or may not be followed by a severe constitutional reaction, depending on the natural resistance of the individual. The severe reactions are caused by the development of a pneumococcus septicemia. which is either temporary, or leads to a fatal termination. The mild reactions are not accompanied by septicemia, and there are no symptoms other than a slight elevation of temperature and moderate leucocytosis. Vaccination with living avirulent pneumococci does not induce severe reactions and is not accompanied by pneumococcus septicemia.

4. Active immunity against pneumococcus pneumonia, produced by vaccination with living pneumococci, appears to be largely independent of the presence or absence of agglutinins and protective bodies in the serum of the monkey.

5. Vaccination with living cultures of Pneumococcus Type I confers against other types of pneumococci a certain amount of cross-immunity which, however, varies considerably with the individual monkey.

6. Immunity against pneumococcus, like other forms of immunity, is a relative term, and depends upon the capacity of the individual for antibody production, the virulence of the invading microorganism, and the size of the dose injected.

STUDIES ON EXPERIMENTAL PNEUMONIA.

VI. Active Immunity Following Experimental Pneumococcus Pneumonia in Monkeys.

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The tendency of certain individuals to repeated attacks of pneumonia has at times given rise to doubt whether acquired immunity to pneumonia exists. Considerable evidence, however, can be brought forward to show that a rather high degree of immunity to the pneumococcus follows an attack of pneumonia. The crisis itself is a striking expression of immunity. Furthermore, Dochez[1] has shown that the serum of patients convalescing from pneumonia usually contains protective substances against the homologous type of pneumococcus, and Blake[2] has demonstrated precipitins in the serum of cases of pneumonia that terminate favorably. Aside from these clinical studies, however, accurate information on the subject of immunity following pneumonia is meager.

The experiments herewith reported were performed for the purpose of testing the degree of active immunity in monkeys subsequent to an attack of experimental pneumococcus pneumonia.

Usually the monkey has first been subjected to a pneumonia of one of the four pneumococcus types; and following this, the resistance to reinfection with a pneumococcus of the same type has been tested. Then, as in the study of vaccination with living pneumococci (Paper V), tests for cross-immunity against one or more of the other types of pneumococcus have been carried out. The same virulent pneumococci were used in these experiments as in the previous studies and the technique used for producing experimental pneumonia was identical with that already described. Philippine monkeys (*Macacus syrichtus*) were employed throughout.

[1] Dochez, A. R., *J. Exp. Med.*, 1912, xvi, 665.

[2] Blake, F. G., *Arch. Int. Med.*, 1918, xxi, 779.

Active Immunity Following Experimental Pneumococcus Type I Pneumonia.

The first experiment was a test for active immunity following experimental Pneumococcus Type I pneumonia. It was shown in Paper I[3] that experimental pneumonia in monkeys is usually fatal when a highly virulent pneumococcus is used for producing the disease. By using very minute infecting doses, however, a certain number of recoveries can be obtained. In a series of *Macacus* monkeys

TABLE I.

Experimental Lobar Pneumonia.

Monkey No.	Weight.	Apr. 29. Broth culture of Pn. I intratracheally.	May 15. X-ray.	Result.
	gm.	*cc.*		
75	4,200	0.0001	Shadow, R. L., L. L.*	Clinical pneumonia. Recovery by crisis on 12th day.
77	2,600	0.000001	Shadow, L. L.	Clinical pneumonia. Recovery by lysis on 11th day.

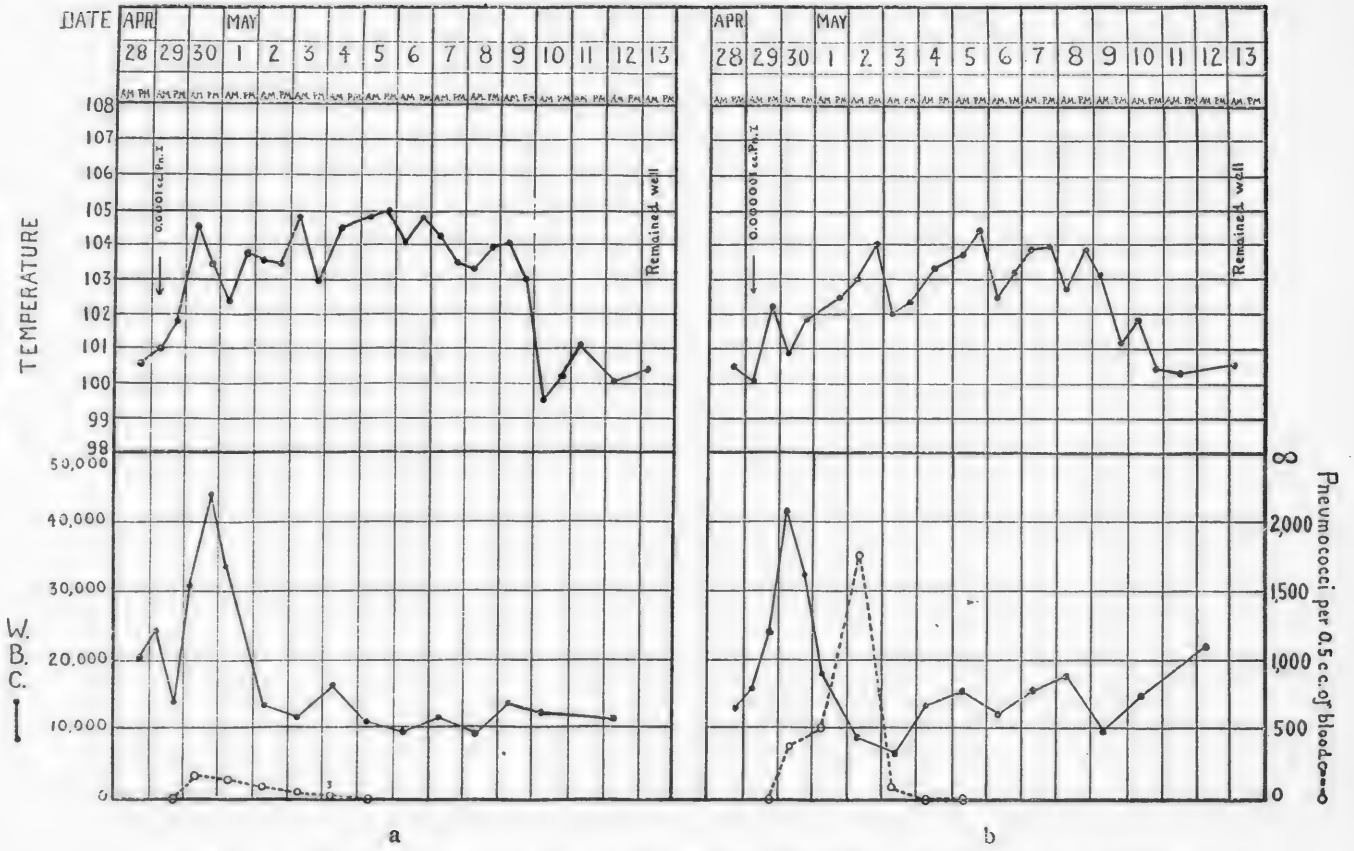
* R. L., R. M., R. U., etc., indicate lobes of the lung. The cardiac lobe is included as part of the right lower lobe.

inoculated intratracheally with a virulent Pneumococcus Type I, two recovered. The following experiment gives the record of these two monkeys.

Experiment 1.—Apr. 29, 1919. Monkey 75 received 0.0001 cc. and Monkey 77, 0.000001 cc. of broth culture of Pneumococcus Type I intratracheally. Both monkeys promptly developed symptoms and signs of lobar pneumonia, with positive blood cultures and sharp leucocyte reactions (Table I, Text-fig. 1). Both monkeys made uneventful recoveries.

2 weeks after the recovery of Monkeys 75 and 77, their blood was tested for agglutinins and protective bodies. Monkey 77 exhibited no agglutinins, but did show some protective bodies; the mouse that received 0.0000001 cc. of culture lived 4 days, and the mouse receiving 0.000001 cc. lived 3 days.

[3] Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, xxxi, 403.



TEXT-FIG. 1, *a* and *b*. Experimental Pneumococcus Type I pneumonia. (*a*) Monkey 75; (*b*) Monkey 77.

TABLE II.

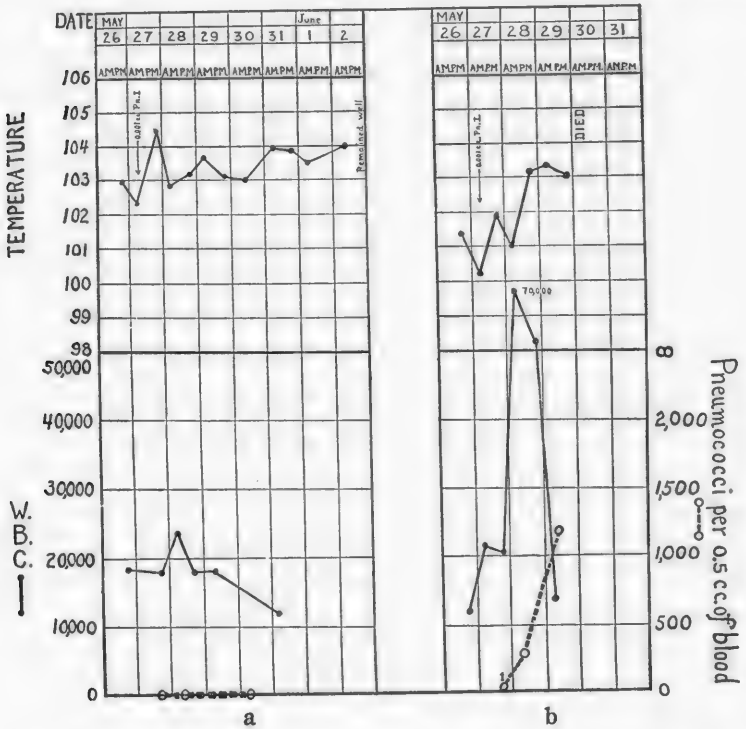
Active Immunity Following Experimental Lobar Pneumonia.

Monkey No.	Weight.	May 27. Serum tests with Pn. I.			May 27. Broth culture of Pn. I intratracheally.	Result.	Autopsy.	Autopsy cultures.		
		Agglutinins.	Protection.	Control.				Lung.	Heart's blood.	Pericardium.
	gm.				*cc.*					
75	4,200	0	0		0.001	Remained well. No signs of pneumonia.				
77	2,600	0	0.00001 cc. D.* 48 hrs. 0.000001 cc. D. 3 days. 0.0000001 cc. D. 4 days.	0.00001 cc. D. 48 hrs. 0.000001 cc. D. 48 hrs. 0.0000001 cc. D. 48 hrs.	0.000001	Remained well. No signs of pneumonia.				
109 (control).	4,585				0.001	Clinical pneumonia. D. 3rd day.	Lobar pneumonia; stage of engorgement, R. U., L. U., R. M., L. M.	Pn. I	Pn. I	
110 (control).	2,710				0.000001	Clinical pneumonia. D. 7th day.	Lobar pneumonia; mixed red and gray stages, entire right lung; acute pericarditis.	" I	" I	Pn. I

* D. indicates died; S., survived.

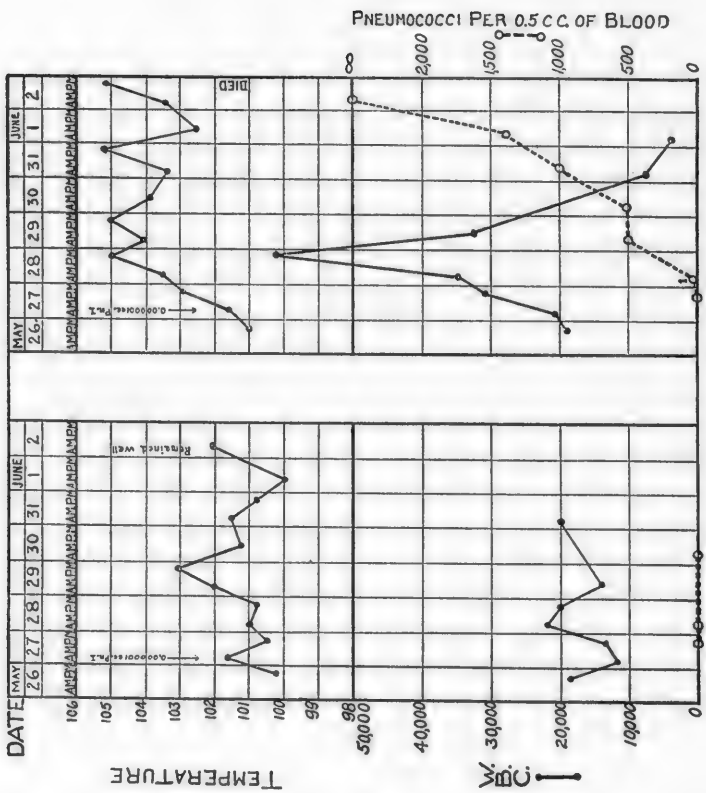
The next step was to test these two monkeys for active immunity against Pneumococcus Type I.

Experiment 2.—May 27, 1919. Nearly 3 weeks after their recovery from pneumonia, Monkeys 75 and 77, with two *Macacus* controls were injected intratracheally with a broth culture of virulent Pneumococcus Type I (the same



TEXT-FIG. 2, *a* and *b*. Active immunity against Pneumococcus Type I following Pneumococcus Type I pneumonia. (*a*) Monkey 75; immunity following pneumonia. (*b*) Monkey 109; control.

strain with which they had been previously infected). Monkey 75 and its control, Monkey 109, each received 0.001 cc. of culture; Monkey 77 and its control, Monkey 110, each received 0.000001 cc. Table II and Text-figs. 2 and 3 show the results obtained.



TEXT-FIG. 3, *a* and *b*. Active immunity against Pneumococcus Type I following Pneumococcus Type I pneumonia. (*a*) Monkey 77; immunity following pneumonia. (*b*) Monkey 110; control.

In Table II it will be seen that the two monkeys which had had a previous attack of Pneumococcus Type I pneumonia remained well, while the two controls developed Pneumococcus Type I pneumonia. Monkey 75 (Text-fig. 2) showed a very slight temporary rise in temperature and leucocytes, but was lively and well and displayed no symptoms whatever of pneumonia. The rather high average temperature was attributed at the time to the hot weather, but subsequently autopsy showed a chronic pulmonary tuberculosis. Monkey 109, the control to Monkey 75, was overwhelmed by the large dose (0.001 cc.) of culture injected and died on the 3rd day of the illness with lobar pneumonia and Pneumococcus Type I septicemia.

Monkey 77 (Text-fig. 3) also remained well and showed very little temperature or leucocyte reaction. The control, Monkey 110, developed lobar pneumonia and died on the 7th day of the disease with a heavy Pneumococcus Type I septicemia.

These experiments corroborate the evidence already at hand in favor of a high degree of active immunity following pneumococcus pneumonia. It will be noted that this immunity existed in Monkey 75 without any evidence of agglutinins or protective substances in the blood. The amount of protective substance in Monkey 77 was almost negligible, yet it also was able to resist reinfection.

Tests for Cross-Immunity Following Pneumococcus Type I Pneumonia.

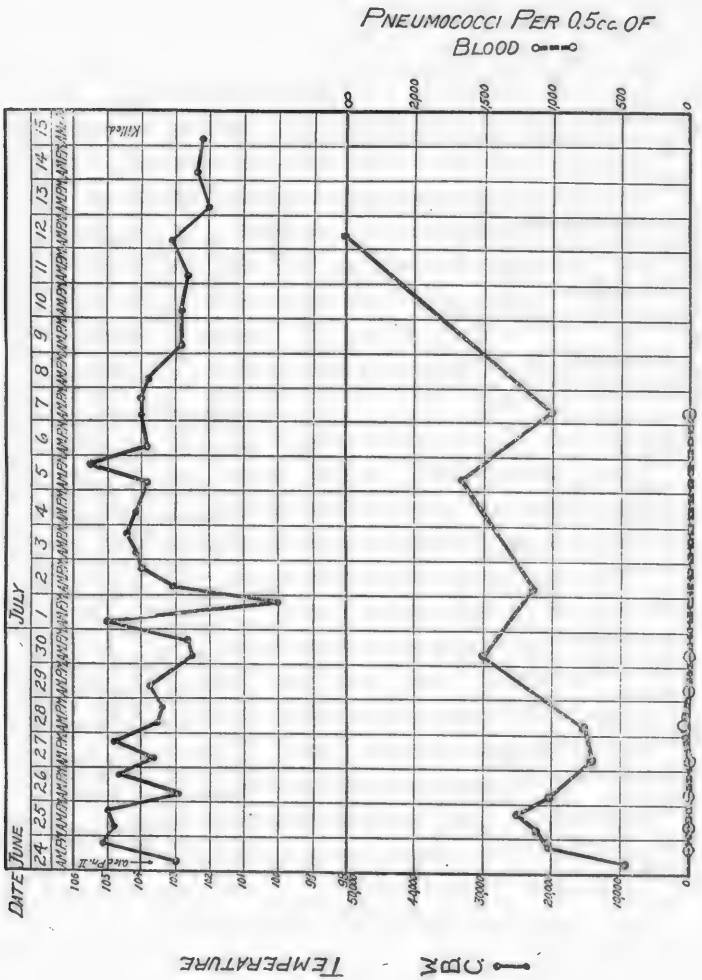
In the preceding paper it has been shown that vaccination with a living culture of Pneumococcus Type I conferred a high degree of immunity against pneumonia of the homologous type; and furthermore, that by such vaccination a certain but variable amount of cross-immunity was also established against the other types of pneumococcus pneumonia. It was therefore desirable to determine whether a similar cross-immunity could be demonstrated in monkeys that had survived an attack of Pneumococcus Type I pneumonia.

Test for Cross-Immunity against Pneumococcus Type II Following Pneumococcus Type I Pneumonia.—In the following experiment one of the monkeys (No. 75) that resisted reinfection with Pneumococcus Type I was tested for cross-immunity against Pneumococcus Type II. The same virulent strain of Pneumococcus Type II was

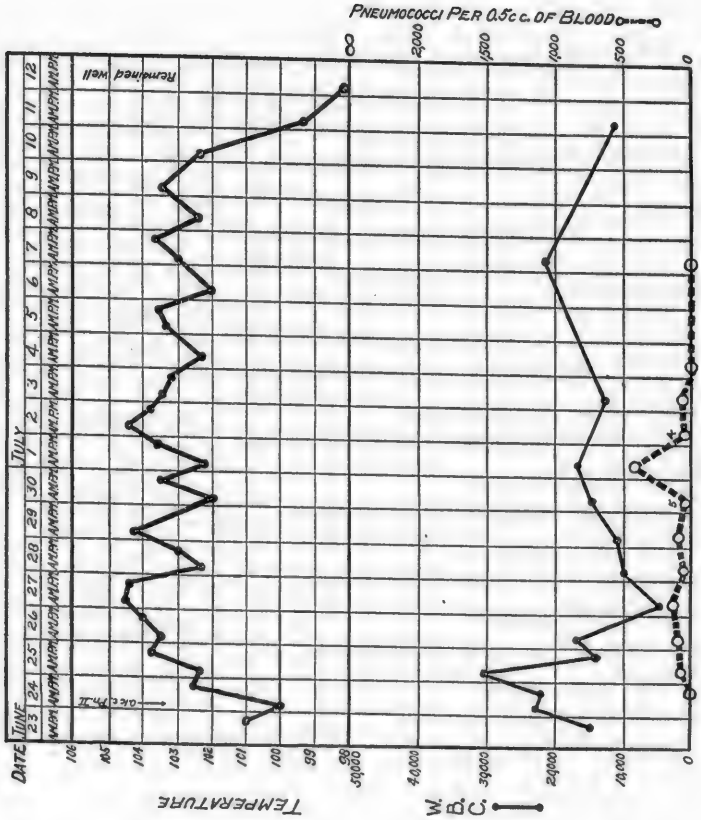
TABLE III.

Pneumococcus Type II Pneumonia Following Pneumococcus Type I Pneumonia.

Monkey No.	Weight.	Apr. 29–May 10.	May 27.	June 23. Serum tests with Pn. II.			June 24. Broth culture of Pn. II intratracheally.	Result.	Autopsy.	Autopsy cultures.	
				Agglutinins.	Protection.	Control.				Lung.	Heart's blood.
	gm.						*cc.*				
75	3,862	Pn. I pneumonia.	Resisted reinfection with Pn. I.	0	0.000001 cc. D. 60 hrs. 0.00001 cc. S. 0.0001 cc. D. 18 hrs. 0.001 cc. D. 18 hrs.	0.000001 cc. D. 48 hrs. 0.00001 cc. D. 20 hrs. 0.0001 cc. D. 18 hrs.	0.1	Clinical pneumonia. Crisis on 8th day. Continuous temperature. Killed on 22nd day.	Chronic pulmonary tuberculosis; general miliary tuberculosis.	No pneumococci.	No pneumococci.
91 (control).	4,002						0.1	Clinical pneumonia. Recovery on 18th day.			



TEXT-FIG. 4. Monkey 75. Pneumococcus Type II pneumonia subsequent to Pneumococcus Type I pneumonia. Pneumococcus Type I pneumonia from Apr. 29 to May 10.



TEXT-FIG. 5. Monkey 91; control for Monkey 75. Experimental Pneumococcus Type II pneumonia.

used in this experiment that had been employed in the vaccination experiments.

Experiment 3.—June 24, 1919. 6 weeks after the attack of Pneumococcus Type I pneumonia, Monkey 75 received 0.1 cc. of broth culture of Pneumococcus Type II intratracheally. Monkey 91 served as control, and received the same dose of culture. The results are shown in Table III and Text-figs. 4 and 5.

Both monkeys developed pneumonia and ran rather unusually long courses with final recovery. Monkey 75, however, continued to run a slight temperature and was killed July 15, the 22nd day after inoculation. Autopsy showed a chronic pulmonary tuberculosis, with early miliary tuberculosis of the various organs. Monkey 91, the control, had had an attack of spontaneous Pneumococcus Type IV pneumonia several months before the experiment, so was not an ideal control. It was the only one available, however, at this time, and had to be used. Ordinarily, an infecting dose as large as the one used in this experiment (0.1 cc.) would have been sufficient to kill a control.

The experiment shows that an attack of Pneumococcus Type I pneumonia did not afford enough cross-immunity to protect Monkey 75 from infection with Pneumococcus Type II. How much of a factor the tuberculosis was in this animal and to what extent it affected the immunity it is impossible to say. The tuberculous process was probably active at the time the immunity against Pneumococcus Type I was tested, for the animal was then running an abnormally high temperature; yet the presence of tuberculosis apparently did not lessen the degree of resistance to Pneumococcus Type I.

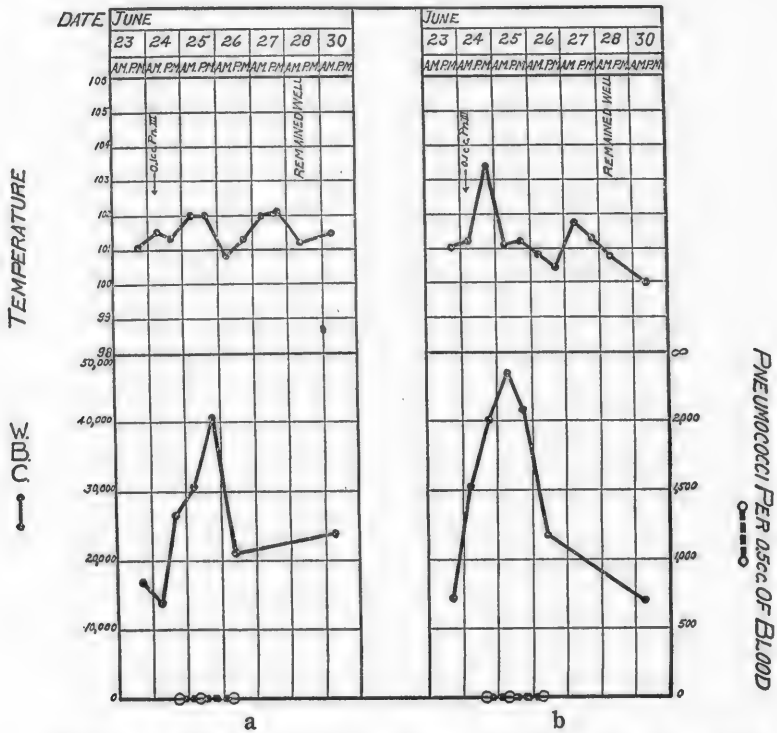
Tests for Cross-Immunity against Pneumococcus Type III Following Pneumococcus Type I Pneumonia.—The next step in this series of experiments was to test a monkey that had recovered from Pneumococcus Type I pneumonia for cross-immunity against Pneumococcus Type III. Monkey 77 was selected for this test.

Experiment 4.—June 24, 1919. 6 weeks after recovery from Pneumococcus Type I pneumonia, Monkey 77 received 0.1 cc. of broth culture of virulent Pneumococcus Type III intratracheally. The control, Monkey 115, received the same dose at the same time. Table IV and Text-fig. 6 show the results obtained.

TABLE IV.

Inoculation with Pneumococcus Type III Following Pneumococcus Type I Pneumonia.

Monkey No.	Weight.	Apr. 29–May 9.	May 27. Broth culture of Pn. I intratracheally.	June 23. Serum tests with Pn. III.		June 24. Broth culture of Pn. III intratracheally.	Result.
				Agglutinins.	Protection.		
	gm.					*cc.*	
77	2,680	Experimental Pn. I pneumonia.	Resisted reinfection with Pn. I.	0	0	0.1	No symptoms of pneumonia.
115 (control).	3,015					0.1	Temporary febrile reaction with accelerated respiration and leucocytosis.



TEXT-FIG. 6, *a* and *b*. Inoculation with Pneumococcus Type III following Pneumococcus Type I pneumonia. (*a*) Monkey 77; Pneumococcus Type I pneumonia from Apr. 29 to May 9. (*b*) Monkey 115; control.

Monkey 77 remained well and had no symptoms whatever except a moderate rise in leucocytes. The temperature of the control monkey rose to 103.4° F. and there was a sharp leucocyte reaction, but the blood culture remained sterile and the monkey did not appear very sick. Evidently the control suffered nothing more than a bronchitis or a very small patch of consolidation.

The Pneumococcus Type III strain used in this experiment at no time proved highly virulent for monkeys, though 0.000001 cc. of a broth culture would invariably kill a mouse. It seemed advisable to repeat this test on another monkey using a larger infecting dose of Pneumococcus Type III. Accordingly, another monkey (No. 96) that had recovered from Pneumococcus Type I pneumonia was tested against Pneumococcus Type III. Monkey 96 had Pneumococcus Type I pneumonia from May 13 to 20.[4]

Experiment 5.—July 1, 1919. Monkey 96, about 6 weeks after the attack of Pneumococcus Type I pneumonia, received 1 cc. of broth culture of Pneumococcus Type III intratracheally (Table V, Text-fig. 7). The control, Monkey 107, likewise received 1 cc. of the same culture intratracheally.

By referring to Table V it will be seen that both monkeys developed a mild interstitial pneumonia, the symptoms of which persisted only 48 hours. Neither monkey presented a positive blood culture at any time. The temperature and leucocyte curves for the two monkeys were strikingly alike.

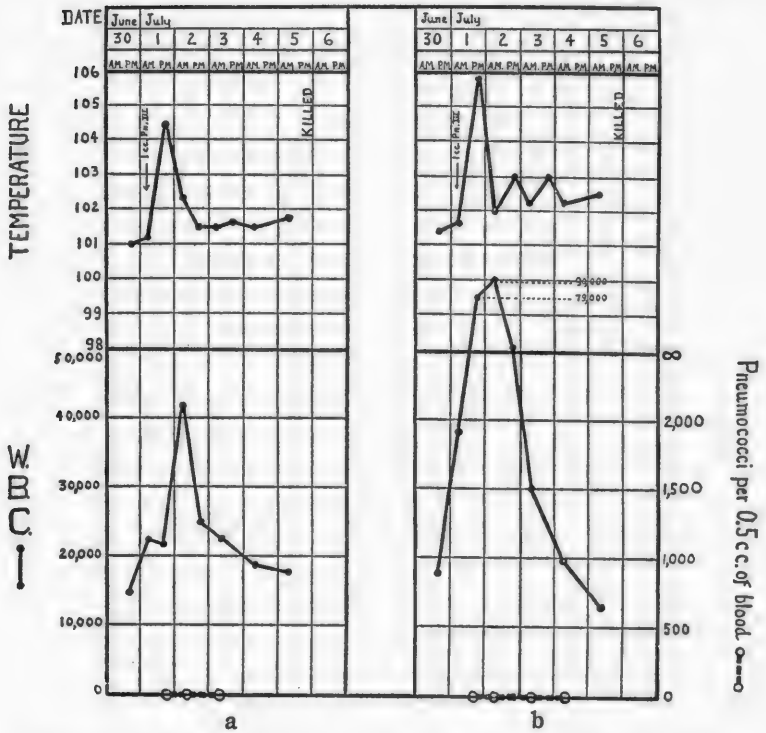
It cannot be maintained that the vaccinated monkey in this experiment showed any definite cross-immunity against Pneumococcus Type III. The disease ran a mild course in both animals for the probable reason that this particular strain of Pneumococcus Type III though virulent for mice possessed only slight virulence for monkeys.

[4] Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, xxxi, 530, Experiment 3.

TABLE V.

Pneumococcus Type III Pneumonia Following Pneumococcus Type I Pneumonia.

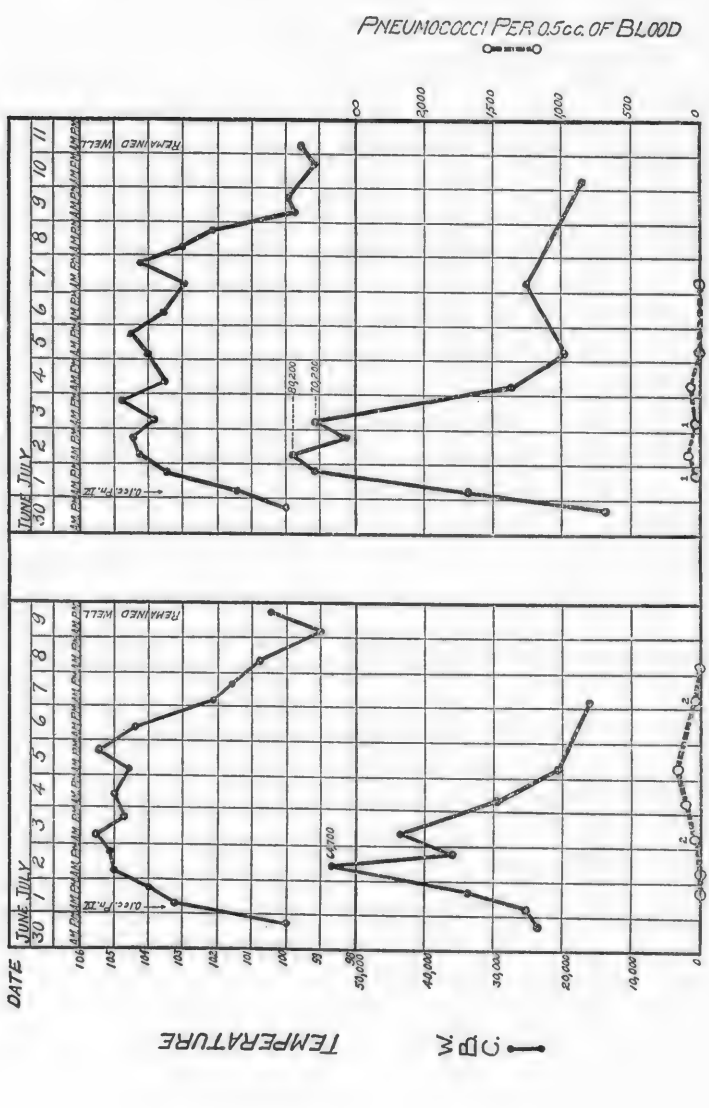
Monkey No.	Weight.	May 13. Broth culture of Pn. I intratracheally.	Result.	July 1. Broth culture of Pn. III intratracheally.	Result.	Autopsy.	Autopsy cultures.	
							Lung.	Heart's blood.
	gm.	*cc.*		*cc.*				
96	2,655	0.000001	Clinical pneumonia. Crisis on 8th day.	1	Very mild pneumonia. Killed on 5th day.	Interstitial pneumonia, R. U., L. U.	No growth.	No growth.
107 (control).	2,334			1	Very mild pneumonia. Killed on 5th day.	Interstitial pneumonia, R. L.	" "	" "



TEXT-FIG. 7, *a* and *b*. Pneumococcus Type III pneumonia subsequent to Pneumococcus Type I pneumonia. (*a*) Monkey 96; Pneumococcus Type I pneumonia from May 13 to 20. (*b*) Monkey 107; control.

Tests for Cross-Immunity against Pneumococcus Type IV Following Pneumococcus Type I Pneumonia.—It has been shown in the previous paper that vaccination with a living Pneumococcus Type I gave no appreciable cross-protection against Pneumococcus Type IV pneumonia. In the present study a somewhat similar test was made for cross-immunity against Pneumococcus Type IV following Pneumococcus Type I pneumonia.

Experiment 6.—July 1, 1919. Monkey 77, 7 weeks after the attack of Pneumococcus Type I pneumonia, and the control, Monkey 115, each received 0.1



TEXT-FIG. 8, *a* and *b*. Pneumococcus Type IV pneumonia subsequent to Pneumococcus Type I pneumonia. (*a*) Monkey 77; Pneumococcus Type I pneumonia from Apr. 29 to May 9. (*b*) Monkey 115; control.

cc. of Pneumococcus Type IV intratracheally (Table VI). This was a virulent pneumococcus isolated from a case of spontaneous lobar pneumonia (Monkey 97) and killed a mouse in doses of 0.000001 cc. of broth culture.

Both monkeys proved susceptible and ran typical courses of lobar pneumonia (Text-fig. 8). Monkey 77 recovered by crisis on the 7th day, Monkey 115 on the 9th day. Both animals had very pronounced leucocyte reactions and positive blood cultures.

These two cases were about equally severe. As both recovered, it is impossible to say whether Monkey 77 had any cross-immunity or not, but the evidence is against such a hypothesis.

TABLE VI.

Inoculation with Pneumococcus Type IV of the Two Monkeys Tested in Experiment 4.

Monkey No.	July 1. Serum tests with Pn. IV (Strain M 97).		July 1. Broth culture of Pn. IV (Strain M 97). intratracheally.	Result.
	Agglutinins.	Protection.		
			cc.	
77	0	0	0.1	Clinical pneumonia. Recovery by crisis on 7th day.
115	0	0	0.1	Clinical pneumonia. Recovery by crisis on 9th day.

Active Immunity Following an Attack of Pneumococcus Type IV Pneumonia.

The so called Pneumococcus Type IV is not a type in the true sense as it consists merely of a large number of pneumococcus strains which do not fall into the three fixed groups and which usually have no biological connection with one another. An animal immunized against one strain of Pneumococcus Type IV develops agglutinins and protective bodies for that particular strain, but as a general rule this serum fails to agglutinate or protect against other Type IV strains.

The question therefore naturally arose whether an attack of Pneumococcus Type IV pneumonia would confer immunity against a second attack by the same strain, and also whether an attack of

Pneumococcus Type IV pneumonia would confer any cross-immunity against other strains of Pneumococcus Type IV.

In order to answer these questions two monkeys that had survived attacks of Pneumococcus Type IV pneumonia were inoculated a second time with Pneumococcus Type IV as shown in the following experiment.

Experiment 7.—July 1, 1919. A large *Macacus*, Monkey 19, 3 weeks after an attack of spontaneous Pneumococcus Type IV pneumonia, was injected intratracheally with 0.1 cc. of broth culture of Pneumococcus Type IV, Strain M 97. At the same time Monkey 111, 3 weeks subsequent to an attack of experimental Pneumococcus Type IV pneumonia (Strain M 97) was also injected intratracheally with 0.1 cc. of broth culture of Pneumococcus Type IV, Strain M 97. Table VII and Text-fig. 9 show the results of this experiment.

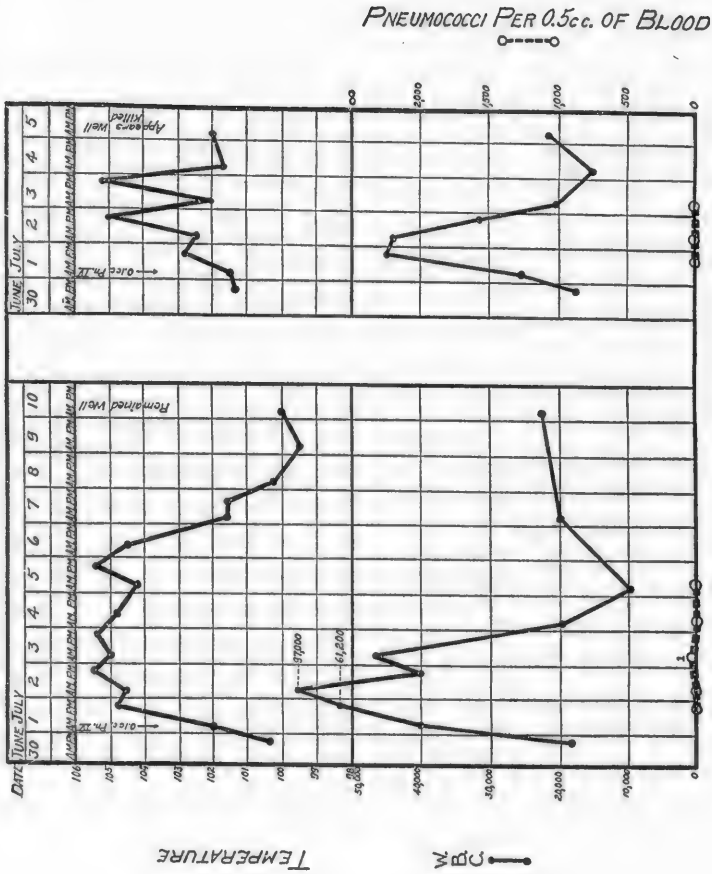
Both monkeys developed Pneumococcus Type IV pneumonia and both recovered. Monkey 19 ran a typical mild course with only one positive blood culture. Monkey 111, inoculated a second time with the identical strain injected 1 month previously, developed an abortive pneumonia and was sick only 2 or 3 days. The blood culture in this animal was never positive.

An unexpected sequel to this experiment presented itself when a biological study was made of the various Pneumococcus Type IV strains that had been isolated during the epidemic of spontaneous pneumonia in the stock monkeys. It was found that the strain originally isolated from Monkey 19 during the attack of spontaneous pneumonia and Strain M 97 were agglutinated by the same Pneumococcus Type IV serum; in other words they belonged to the same biological group. The experiment had been planned to test the amount of immunity against Pneumococcus Type IV following an attack of Pneumococcus Type IV pneumonia. Monkey 19 was to be tested for cross-immunity against a supposedly heterologous strain, Monkey 111 for active immunity against the homologous strain. But since the pneumococcus isolated from Monkey 19 during its original attack of spontaneous pneumonia and Strain M 97 belong to the same subgroup of Pneumococcus Type IV, it is apparent that in both monkeys we were testing for active immunity against the homologous strain of Pneumococcus Type IV. Strangely enough both monkeys failed to show sufficient immunity to protect them against reinfection with

TABLE VII.

Reinfection with Pneumococcus Type IV Following an Attack of Pneumococcus Type IV Pneumonia.

Monkey No.	Weight.	Lobar pneumonia Pn. IV.	July 1. Serum tests with Pn. IV (Strain M 97).		July 1. Broth culture of Pn. IV (Strain M 97) intratracheally.	Result.	Autopsy.	Autopsy cultures.	
			Agglutinins.	Protection.				Lung.	Heart's blood.
	gm.				*cc.*				
19	4,000	June 1–8. Spontaneous (Strain M 19).	0	0	0.1	Clinical pneumonia. Recovery by crisis on 7th day.			
111	1,670	May 27–June 9. Experimental (Strain M 97).	0	0	0.1	Temporary febrile reaction and leucocytosis. Killed on 5th day.	Old unresolved pneumonia, R. U. Patch of fresh infection (engorgement), R. L.	R. L., Pn. IV.	No growth.



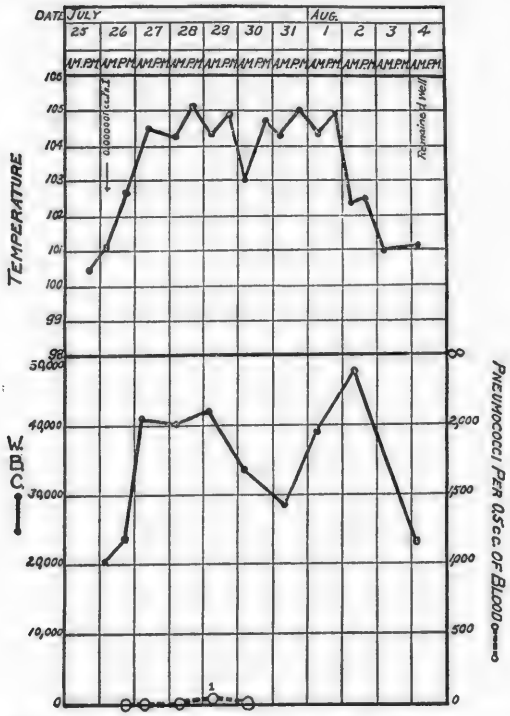
TEXT-FIG. 9, *a* and *b*. Pneumococcus Type IV pneumonia subsequent to Pneumococcus Type IV pneumonia. (*a*) Monkey 19; spontaneous Pneumococcus Type IV pneumonia from June 1 to 8. (*b*) Monkey 111; experimental Pneumococcus Type IV pneumonia (Strain M 97) from May 27 to June 9.

the same strain of Pneumococcus Type IV which had excited in them an attack of pneumonia a month previously. But although these monkeys were unable to resist reinfection with Pneumococcus Type IV, it is clear that one of them, Monkey 111, had a considerable amount of active immunity—enough to reduce the second attack to a very mild, abortive pneumonia. Furthermore, it is worthy of note that the autopsy on this monkey showed that it had never recovered completely from the first attack. An old unresolved pneumonia was found in the right upper lobe, while the fresh infection was limited to the right lower lobe.

Test for Cross-Immunity against Pneumococcus Type I Following Pneumococcus Type IV Pneumonia.—The last experiment in this series was undertaken to determine whether an attack of Pneumococcus Type IV pneumonia would confer any cross-immunity against Pneumococcus Type I pneumonia.

Experiment 8.—Monkey 115. *Macacus syrichtus*, female; weight, 3,015 gm. July 1, 1919. Intratracheal injection of 0.1 cc. of 18 hour broth culture of Pneumococcus Type IV, Strain M 97. July 2. Symptoms of pneumonia. Blood culture positive, Pneumococcus Type IV. July 9. Crisis and recovery. July 26. Intratracheal injection of 0.000001 cc. of 18 hour broth culture of Pneumococcus Type I. July 27. Symptoms of pneumonia. July 29. Blood culture positive (one colony of Pneumococcus Type I in 0.5 cc. of blood). Aug. 2. Crisis and recovery. The results are shown in Text-fig. 10.

This experiment is in line with the other experiments on cross-immunity. The monkey was not sufficiently immune to resist infection with Pneumococcus Type I, but, in view of the mild course and the practically sterile blood culture, it would appear that enough cross-immunity was present to modify favorably the course of the disease. Certainly, if an attack of Pneumococcus Type IV pneumonia does not stimulate enough protection to prevent a second infection by the same strain, it could hardly be expected to elaborate an adequate cross-immunity against a highly virulent Pneumococcus Type I.



TEXT-FIG. 10. Monkey 115. Pneumococcus Type I pneumonia subsequent to Pneumococcus Type IV pneumonia. Pneumococcus Type IV pneumonia from July 1 to 9.

DISCUSSION.

The experiments which have been reported prove conclusively that in monkeys an attack of Pneumococcus Type I pneumonia protects against a second infection by the homologous type. It would be desirable to know how long this immunity lasts, but circumstances have not as yet afforded an opportunity for settling this point.

An attack of Pneumococcus Type I pneumonia gave little if any cross-protection against Pneumococcus Type II pneumonia. The experiments with Pneumococcus Type III were not altogether satisfactory, as the strain employed was not particularly virulent for

monkeys. In both the Type II and Type III experiments, however, rather large infecting doses were employed, 0.1 cc. of Pneumococcus Type II and 0.1 to 1 cc. of Pneumococcus Type III. As in vaccination with living cultures, a small amount of cross-immunity against the other fixed types of pneumococcus is probably always present for a certain length of time following experimental Pneumococcus Type I pneumonia. As in vaccination with living cultures, however, the degree of cross-immunity depends to some extent on individual variation. The virulence and dose of the invading microorganism are, of course, other factors of great importance. An attack of Pneumococcus Type I pneumonia does not appear to induce any higher degree of cross-immunity than vaccination with a living Pneumococcus Type I affords.

It is impossible to say why Pneumococcus Type IV pneumonia fails to protect the monkey against a second infection by the homologous strain when Pneumococcus Type I confers excellent immunity against reinfection. The highly parasitic character of Pneumococcus Type I may account for this difference. At any rate, these observations offer little hope for a satisfactory prophylactic vaccine against Pneumococcus Type IV pneumonia.

From the information obtained in this study it would appear possible that individuals who suffer from repeated attacks of pneumonia are being reinfected from time to time with Pneumococcus Type IV. Further evidence in favor of such a theory is afforded by the fact that in cases of this kind the disease usually runs a mild course.

Attention is again invited to the inconsistency between tests for protective bodies against the pneumococcus in the serum of monkeys and tests for active immunity against pneumonia in these animals. In this respect the results are in harmony with those reported in Papers IV[5] and V where striking disagreements were sometimes noted in the two methods for testing immunity. In the present study, for example, Monkey 75 showed no agglutinins or protective bodies against Pneumococcus Type I in the blood subsequent to the attack of Pneumococcus Type I pneumonia; yet when reinjected with Pneumococcus Type I intratracheally, this animal possessed a sufficient degree of active immunity to resist reinfection.

[5] Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, xxxi, 519.

CONCLUSIONS.

1. Experimental Pneumococcus Type I pneumonia in monkeys confers on them an immunity which protects them against subsequent infection with the homologous type of pneumococcus. The duration of this immunity has not been determined.

2. A certain amount of cross-immunity against the other fixed types of pneumococcus pneumonia may or may not be present following experimental Pneumococcus Type I pneumonia. The degree of cross-immunity is difficult to measure and probably varies widely with the individual monkey.

3. Experimental Pneumococcus Type IV pneumonia in monkeys confers slight if any protection against subsequent infection with the same, or with an homologous strain of Pneumococcus Type IV.

4. There is no evidence in monkeys of cross-immunity against Pneumococcus Type IV pneumonia following Pneumococcus Type I pneumonia; and conversely, Pneumococcus Type IV pneumonia confers no cross-immunity against Pneumococcus Type I pneumonia.

EXPERIMENTAL SYPHILIS IN THE RABBIT.

II. Primary Infection in the Scrotum.
Part 1. Reaction to Infection.

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Plates 72 to 82.

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Primary skin lesions in the rabbit were first reported in 1908. Hoffmann, Löhe, and Mulzer (1), and Ossola (2) reported instances of scrotal infection resulting from testicular inoculation, while Levaditi and Yamanouchi (3) succeeded in producing infection by inoculation of the prepuce. With the introduction of skin inoculations, a decided advantage was gained in the study of experimental syphilis. Not only were the conditions of the inoculation more akin to the mode of infection in the human subject, but the lesions themselves were more analogous to the primary lesions of man. In addition, the infection took place in a region more accessible to observation and the reaction to inoculation could be followed directly, which in itself was a decided advantage.

Of the various skin areas in which inoculation was attempted, the scrotum proved to be best adapted and is now the only area used for purposes of routine inoculation. The methods devised for scrotal inoculation were of three types, all of which were quite simple and consisted in the implantation of bits of infected tissue beneath the skin of the scrotum, in the injection of a fluid medium containing the infecting organisms, or in scarification of a skin area and the local application of the virus. Satisfactory results have been reported from the use of all three of these methods.

EXPERIMENTAL.

In our own work upon scrotal infections of *Treponema pallidum*, the experimental conditions employed were much the same as those outlined in the case of the testicular infections (4).

Animals Used.—A great variety of animals was used, but those preferred were young albinos, grays, browns, or Dutch belts. Individual animals were chosen in which the scrotum was thin and delicate and as free from hair as possible. Old animals with thick, fibrous, or redundant scrota gave results inferior to those of younger animals with more active tissue.

Method of Inoculation.—Inoculation of the scrotum was carried out according to one of two methods. The procedure commonly employed was essentially the same as that described by Tomasczewski (5) which consists in the implantation of a small piece of infected tissue in a pouch prepared for it in the scrotum. The tissue used was either an infected testicle or a granulomatous skin nodule removed with as little contamination as possible and cut into pieces measuring from 3 to 5 mm. in diameter. The inoculation was performed either by the use of a trocar needle of 8 to 9 gauge inside diameter or by snipping the superficial layers of the scrotum with scissors and introducing the fragment of tissue by means of a pair of forceps; the latter method was in general preferred. The only precautions necessary were the observance of reasonable cleanliness and making the implants as superficial as possible.

Both of these methods proved highly satisfactory when rapid development of large skin lesions was desired. They are not adapted, however, to the study of the finer details of the reaction to infection, and for this purpose, we used an intracutaneous or subcutaneous injection of a virus emulsion prepared as for testicular inoculation except that the emulsion contained a greater number of spirochetes, averaging 5 to 10 to the microscopic field. In this operation, the scrotum was cleansed as usual and 0.1 to 0.2 cc. of the emulsion injected by inserting the needle well above the point to be inoculated and carrying it downward to the desired location.

Material Studied.—During the past 4 years, we have inoculated a large number of rabbits by these two methods. Among the earlier animals, there was an occasional one in which infection could not be established with absolute certainty, but all animals inoculated during the last 3 years have developed characteristic lesions from which *Treponema pallidum* could be recovered.

The material afforded by these rabbits was used in various ways. After well developed lesions had become established, a majority of them were used for therapeutic experiments and hence were not available for further study of the course of the local infection except where recurrent lesions developed. Some lesions were excised for histological study at various stages of their development, but a large number of rabbits was held under constant observation throughout the course of the local infection,—some of them as long as 18 months and a few for 2 years or longer.

Reaction Following Inoculation with a Virus Emulsion.

By whatever method scrotal inoculation is performed, the specific reaction which takes place is essentially the same. The early stages of the reaction can be traced more accurately, however, by the use of some methods than by others, and for this purpose, we used an emulsified virus which is but little more than a diluted tissue extract containing the *pallidum*. When inoculations are properly performed by this method, there is very little traumatism, and the slight inflammatory reaction which may develop on this account disappears completely within 24 to 48 hours. Thus, one is not only able to keep the dose of virus used comparatively uniform and to control the conditions of inoculation, but the resulting reaction is as little complicated by extraneous circumstances as is practicable upon any large scale. The chief objection to the method is that it tends to diffuse the infection over a wider area and the resulting reaction is not always so sharply circumscribed as one might wish.

Incubation Period.

The incubation period of scrotal infections produced by inoculation with a virus emulsion came within the comparatively narrow limits of 1 and 4 weeks. Under favorable circumstances, a definite reaction was usually recognized within 10 days to 2 weeks after inoculation and in many instances by the end of the 1st week. Occasionally, the specific reaction developed more slowly and characteristic lesions were not recognizable for several weeks.

The average incubation period of scrotal infections was somewhat shorter than that previously given for the development of gross or clinical alterations in the testicle but corresponded fairly well with

the time within which lesions could be demonstrated microscopically or within which multiplication of spirochetes could be determined by dark-field examination of fluid obtained from the testicles.

Form of the Initial Reaction.

The specific reaction in the scrotum appeared in one of three general forms differing somewhat according to the location of the lesion. The first and most important form of reaction began as small circumscribed swellings or diffusely spreading patches of a rose-pink color situated in the papillary and reticular layers of the skin. These lesions were soft or gelatinous in character and were frequently associated with a rich vascular network such as that shown in Fig. 1, which is probably the most significant feature of the syphilitic reaction. The second form taken by the initial lesion was that of a minute translucent nodule or plaque of induration likewise situated in the papillary layer of the skin but more superficial than the first (Fig. 5). The third type of lesion appeared as an opaque porcelain-white nodule or plaque of extreme hardness situated in the depths of the scrotum and was usually connected with the outer surface of the tunica vaginalis. The first two forms of reaction were the most common and usually the earliest to appear.

Development of the Primary Lesions, or Chancres.

The development of primary skin lesions, or chancres, from initial foci of reaction, such as those described, followed a course which may be represented in general by Figs. 1 to 4 and 5 to 8 which were from two animals of the same series. As indicated in these illustrations, the progress of the infection was marked by an extension of the reaction and the occurrence of certain transformations. The infection tended to spread diffusely through the scrotum, and as the reaction about the focus of infection increased, lesions were formed which assumed the character of circumscribed nodular elevations or of flattened, diffusely spreading patches.

In the development of the gelatinous type of lesion (Figs. 1 to 4), the swelling first subsided to a slight extent and the color changed to a more coppery tint. The lesions then became firmer, and as the

induration increased, changed to more opaque, porcelain-white masses. Purplish red spots and streaks of congestion and hemorrhage then appeared over the central portions of these lesions and with them yellowish gray or yellowish brown areas of necrosis which tended to spread and become covered with thin scales or crusts. Finally, the central area of the lesion became necrotic and sloughed away or was covered by a more or less continuous crust.

In the case of circumscribed lesions such as those in Figs. 1 to 4, these changes led to the formation of elevated masses with a necrotic or ulcerated center surrounded by a zone or collar of induration. If the lesions spread diffusely, as they not infrequently did, the resulting lesion was a thickened or indurated patch over which the areas of necrosis were more diffusely scattered; the crusts were usually imperfectly developed and loosely attached, and while ulceration was slight, weeping patches were formed here and there.

When the scrotal lesions first appeared as translucent nodules or patches of induration, development of the lesions followed a slightly different course (Figs. 5 to 8). The vascular reaction about such areas was relatively less than in the preceding case. As the lesions increased in size, the central portions became more dense and opaque, while the overlying skin became smooth and glistening. The covering epithelium was thinned out until small defects or superficial areas of necrosis appeared and were covered by thin scabs or crusts.

When these lesions assumed the form of circumscribed nodules (Figs. 5 to 8), the area of necrosis enlarged and deepened with the growth of the lesion and was covered by a thick crust or formed a depressed ulcer surrounded by a mass of indurated tissue. The more diffuse lesions of this class appeared as parchment-like thickenings in the skin which either remained intact and appeared translucent, smooth, and glistening throughout or were irregularly covered by loosely attached scales with raw or ulcerated areas here and there.[1]

The third form taken by the initial lesion in the scrotum, that of an opaque white nodule or plaque of induration in the depths of the scrotum, usually followed a still different course of development.

[1] See section below on diffuse scrotal lesions.

As in the case of the lesions just described, the vascular reaction about these lesions was of minor degree. They developed rather slowly as a rule and grew or spread in the form of extremely hard masses over which the skin was freely movable. The plaques usually remained deep seated and spread along the surface of the tunica vaginalis, but the nodular lesions gradually extended towards the surface so that the overlying skin not infrequently became involved. Necrosis and ulceration then took place with the formation of lesions much like those which have been described. However, many of these deep seated nodules did not involve the overlying tissues to a sufficient extent to bring about necrosis and ulceration but remained throughout as circumscribed nodular lesions in the deeper tissues of the scrotum.

The subsequent course of the reaction to infections produced in this way was essentially the same as that of an infection produced by other methods of inoculation and may be followed out in connection with the infection produced by implantation.

Reaction Following Inoculation by Tissue Implantation.

While inoculation by the use of tissue implants possesses certain advantages, there may be some difficulties attending its use. Due to unequal distribution of spirochetes in infected tissues as well as to inequalities in the vitality of the organisms present in different portions of a lesion, inoculations carried out by implantation may not yield results which are as uniform as from the use of methods by which the dose of virus can be kept more nearly constant. It is possible, however, to overcome these difficulties to a large extent by the observance of certain precautions: first, the use of infected tissues at a relatively early stage of the infection or only at a period when the infection is actively progressing; second, the use of only such portions of a lesion as are actively growing at the time; and third, as a means of insuring an adequate dose of organisms when any doubt exists, the use of pieces of tissue as large as can conveniently be used without causing necrosis and sloughing of the surrounding tissues. Obviously, judgment in the use of these precautions can be acquired only by experience. A second difficulty in the

use of this method is the traumatism necessarily inflicted and the chances offered for the development of secondary infections. These need not prove serious, however, since reasonable care in the performance of inoculations will remove such difficulties to a considerable extent.

For certain purposes, implantation has advantages over other methods of inoculation which more than compensate for its disadvantages. When properly safeguarded, large, actively growing skin lesions can be obtained more quickly and with greater certainty by this method than by any other method of inoculation with which we are acquainted.

Incubation Period.

The exact incubation period of infections produced by scrotal implantation is difficult to fix since a non-specific foreign body reaction frequently overshadows the specific reaction. As nearly as can be determined, however, the incubation period in our series of animals fell within much the same limits as for inoculations made with emulsions. There was usually a definite specific reaction about the implant, and spirochetes could be obtained from the surrounding tissues within 10 days to 2 weeks after inoculation and were obtained as early as the 3rd day, but it was not certain that the organisms found were more than transient invaders. In a few instances, incubation was unusually prolonged after inoculations performed by this method, and no specific reaction could be recognized for several months—in one instance 6 months and 6 days. This unusual prolongation of the incubation period probably had no connection with the method of inoculation used except as a possible instance of the inoculation of a small number of organisms or of organisms of low vitality, and is merely cited to show the time which may elapse before any definite reaction can be detected.

Early Reaction.

The reaction which takes place in the scrotum following inoculation by implantation may be regarded as partly one of wound healing, partly a foreign body reaction, and partly specific in character. During the first 24 hours, the scrotum became slightly reddened and

edematous, but this acute inflammatory reaction rarely lasted beyond the 2nd or 3rd day. Then the process of organization became established; the swelling subsided, leaving the skin about the implant slightly reddened, smooth, and glistening. This condition persisted for upwards of 7 to 9 days without producing any considerable increase in the size of the nodule. In some instances, the skin over the implant became necrotic and was covered by a crust or sloughed away leaving a depressed ulcer surrounded by a zone of granulation tissue. If no specific reaction had developed by this time, the nodule began to shrink and soften while the skin became relaxed as the process of organization or healing subsided. If the tissues about the implant retained their appearance of activity beyond the 10 day period, and especially if the reaction appeared to be increasing, it was usually found that a specific reaction had commenced.

However, the specific reaction usually became apparent after the process of organization had abated to an appreciable extent and appeared almost as a renewal of this process. The differences which could be recognized were that there was less reddening in the specific reaction, the tissues were more translucent and more definitely indurated, and the reaction resulted in a rapid growth of the lesions. In many instances, there was no break in the continuity of the two processes and it was impossible to determine exactly when the specific reaction began.

Not infrequently, multiple foci of infection resulted from inoculation by this method. Thus, lesions developed independently about the implant at the point of incision and occasionally other discrete foci were scattered along the track of the sinus.

Course of the Scrotal Reaction and Development of Typical Scrotal Chancres.

In following out the successive stages in the scrotal reaction and the development of what may be called typical scrotal chancres, it may be assumed that the specific element in the reaction is the same in all essential respects whether inoculation is carried out by the use of a virus emulsion or by tissue implantation. The mechanical conditions of the reaction in the two cases are quite different, however.

The production of an infection by the introduction of a solid mass of tissue into the scrotum undoubtedly exercises a considerable influence in itself upon the character of the lesions produced and favors the development of large circumscribed lesions, but chancres produced in this way do not always follow the same course of development, and the resulting lesions themselves may be quite different. It is well, therefore, to consider the growth of these lesions according to the general character of the reaction.

Three characteristic examples of chancre development following the use of this mode of inoculation are given in Figs. 9 to 12, 13 to 16, and 17 to 20. The first group of photographs (Figs. 9 to 12) represents stages of chancre development where optimum conditions of virus, animals, and technique were combined. The animal shown was one of a series of ten rabbits in which the results were remarkably uniform. The first photograph (Fig. 9) taken 10 days after inoculation shows a well established specific reaction. There were some edema and congestion of the scrotum, and the skin over the implants had undergone necrosis with the formation of hard dry crusts which showed a line of demarcation at their periphery. Both implants were surrounded by a distinct but narrow zone of specific granulation, more prominent on the left than on the right. These lesions developed very rapidly and by the 13th day (Fig. 10) presented all the characteristics of typical chancres with depressed ulcers surrounded by broad zones of induration.

With the growth of the lesions as illustrated in Figs. 11 and 12, taken 3 and 5 weeks respectively after inoculation, the diffuse congestion and edema of the scrotum persisted; the scrotum gradually became thickened and a few petechial hemorrhages appeared towards the lower end of the right scrotum (Fig. 11), which formed the center about which a second circumscribed lesion soon developed. Eventually, the entire scrotum of both testicles became involved in the specific reaction and the condition existing at the end of the 5th week was that shown in Fig. 12. On the right, there were two circumscribed lesions connected by areas of more diffuse infiltration, while on the left, there was a single massive chancre surrounded by tissues which were themselves markedly infiltrated and covered by diffusely spreading patches of hemorrhage and necrosis.

This is an instance of a high grade infection in the scrotum of the rabbit such as may be produced under favorable conditions and has been observed many times. It may not be out of place to point out that this case illustrates, in particular, first the extreme rapidity with which the infection may become established and typical circumscribed lesions developed, and secondly the inherent tendency of

pallidum infections to spread beyond the local confines of the initial focus of infection, together with something of the character of the reaction which occurs under such circumstances.

The second case in this group (Figs. 13 to 16) illustrates a course of chancre development which is more nearly an average than that just described. The reaction exhibited by this animal was of the same general character as that of the first, but differed in two essential respects. There was early ulceration of the lesions as in the former case, and by the 14th day, characteristic chancres with well defined collars of induration were present (Fig. 13). The growth of the lesions then ceased, the edema in the scrotum subsided, and the chancres themselves decreased in size (Fig. 14). During the 4th week, there were a slight recurrence of the edema (Fig. 15) and renewed growth of the circumscribed lesions which continued with slight remissions until large indurated lesions were formed (Fig. 16, 57 days after inoculation).

These two examples will serve to illustrate the type of reaction which takes place in most instances of active chancre development. A third form of reaction commonly seen is that illustrated in Figs. 17 to 20. This animal was one with a rather thick fibrous scrotum (note the scrotum in Figs. 17 and 18) and the implants could not be made so superficially as one would wish. The incubation period was somewhat longer, and the lesions did not begin to grow actively until about the end of the 4th week after inoculation. The photograph reproduced in Fig. 17 was taken at the end of the 5th week (34 days) when the nodules measured approximately 1 cm. in diameter. The skin about the nodules was still but little affected and for the most part retained its normal appearance. During the next 18 days, these lesions underwent a considerable transformation (Figs. 18 to 20); they increased in size and became more indurated, while the process extended to the overlying skin which became smooth, translucent, and highly refractile. Areas of necrosis then appeared upon the surface, and as these extended and contracted, the lesions flattened out into the form seen in Fig. 20. Specific reactions of this type were especially apt to occur when the lesions developed in the depths of the scrotum or when, for any reason, the reaction pursued a less active course and involvement of the skin with consequent necrosis and ulceration took place at a relatively late period in the growth of the lesions.

Multiple Chancres.—Multiple lesions of a chancre-like character developed in the scrotum under two different conditions, first as a result of simultaneous infection of several points in the scrotum, and second as a result of spread of the infection from a given focus. While lesions of both classes might be regarded as chancres, the significance of the two cases is different, and in the present connection, we shall refer only to lesions of the first class.

Inoculation of the scrotum of the rabbit by the methods described usually resulted in the production of unicentric lesions. Not infrequently, however, lesions appeared simultaneously at other points reached directly in the process of inoculation. The most common seat of the accessory chancre was the point of incision in the skin, and a typical instance of an early lesion of this kind is shown in Fig. 21, the photograph of which was taken 14 days after inoculation. Accessory lesions of this type were frequently abortive or were overgrown by the more vigorous reaction about the main focus of infection as was the case in this animal (Fig. 22, 36 days after inoculation).

A second case of a similar character is shown in Fig. 23. Originally there was an accessory focus of reaction on both sides of this animal. That in the right scrotum was abortive and had almost disappeared when this photograph was taken (29 days after inoculation), while the one on the left was growing quite actively.

In some animals the accessory chancres reach a considerable size even though they are encroached upon by the main chancre mass. Figs. 24 and 25 show a case of multiple chancres in which there were three foci of reaction in the right scrotum and two in the left (Fig. 24, 29 days after inoculation). As these lesions developed, the middle focus of infection on the right became obliterated, but the other accessory chancre grew almost as vigorously as the main lesion. On the left, the two lesions fused at their proximal borders forming a figure of eight chancre (Fig. 25, 49 days after inoculation).

In rare instances, one finds little difference in the vigor displayed by the several lesions present, and all may develop at about an equal rate as shown in Fig. 26 (47 days after inoculation). In addition to the group of multiple lesions described, all of which were situated in the skin itself or pointed upon the skin surface, there was another interesting group of multiple focal lesions in which the foci of reaction were located at different levels in the scrotum—one above the other; these lesions were situated in the skin and upon the outer surface of the tunica vaginalis. This form of localization occurred especially after subcutaneous inoculation with a virus emulsion and is of especial interest in revealing a tendency of *Treponema pallidum* to localize in these two structures.

These few examples of the reaction to infection in the scrotum and the growth of the primary lesions will serve to illustrate what may be called typical skin reactions and typical chancre development in the sense that they represent processes which are in themselves perfectly characteristic and result in the formation of lesions which are easily recognized as lesions of a specific character. In many instances, however, reactions occur and lesions develop which are not so well recognized as processes of a syphilitic nature, and we may refer briefly to a few instances of this kind.

Influence of Different Elements of the Specific Reaction upon Chancre Development.

Irregularities of Chancre Development.

The condition which gives the characteristic picture to the specific reaction in the skin and to the chancre itself is the maintenance of a certain balance among the several processes which take part in this reaction. It was found that when for any reason this balance was disturbed, the character of the reaction was altered and the lesions became modified in accordance with existing conditions. The irregularities of chancre development which were most commonly seen were associated with the occurrence of excessive edema or of congestion and edema, with conditions simulating an acute inflammatory reaction, or with irregularities in the process of granulation, the cause of which cannot be discussed.

Edema.—It was found that excessive edema with or without congestion might occur at any time during the course of the local infection. When it developed at an early stage of the infection, it not infrequently overshadowed the proliferative reaction and produced a lesion which showed chiefly a depressed ulcer with a firm margin surrounded by an edematous skin (Fig. 27). In some instances, this condition persisted for some time, while in others it was no more than an important feature of the reaction or an event in the course of the reaction. Figs. 28 and 29 illustrate an instance of this kind in which the specific reaction was proceeding rather slowly, and at the end of the 3rd week, there were ulcers surrounded by a narrow zone of induration. An acute edema then developed (Fig. 28), and with the appearance of this edema, the focal lesions began to grow at an extremely rapid rate; in 2 weeks, they had reached the condition shown in Fig. 29, the edema persisting to an appreciable extent all the while.

Late edema or edema occurring after characteristic lesions had developed was also seen occasionally and was so marked in some instances as to obscure completely the character of the lesion (Fig. 30).

Acute Inflammatory Reactions.—A small number of rabbits in the series showed an early reaction resembling an acute inflammatory process. There was the usual inflammatory reaction during the first few days following inoculation, and as this subsided, an extremely active process of granulation set in; the tissues surrounding the implant remained reddened or cyanotic and were quite firm, while the zone of induration increased rapidly. The center of the nodule became necrotic and sloughed, leaving a moist, depressed ulcer, or, if no slough occurred, the necrotic tissue softened to a creamy semifluid mass covered by a crust. As the reaction progressed, the tissues surrounding the zone of granulation or even

the entire scrotum became congested and edematous (Fig. 31). This condition usually lasted for not more than 3 to 5 weeks before regression set in. The lesions which appeared subsequently were usually of a minor character, but in some instances, typical chancres were formed as in the case illustrated (Figs. 31 and 32).

At first we were inclined to regard these acute reactions as non-specific, inflammatory processes, but investigation showed that this was not entirely correct. Spirochetes were present in considerable numbers both in the zone of granulation and in the edematous portions of the scrotum entirely removed from the region of the implant, but secondary infection could not be excluded as a factor in the reaction. However, true suppuration did not occur as it usually did where pyogenic organisms were present to any considerable extent. It was noted that these reactions were more frequent when large implants were made from testicles during an early stage of extremely active infections. It seemed possible, therefore, that these were cases of acute specific reaction influenced to a greater or less extent by bacterial infection as well as by the implant itself.[2]

Irregularities in the Process of Granulation.—In many instances, irregularities in the development of scrotal chancres were traceable to peculiarities in the process of granulation, and since this is such a noticeable feature of what we are accustomed to regard as the typical *pallidum* reaction, the peculiarities naturally took the form of a lowered intensity of reaction or of some irregularity in the process, both of which were commonly seen in scrotal infections in the rabbit.

The case illustrated in Figs. 33 to 36 furnishes an example of an abortive skin reaction with subsequent development of granulomatous nodules in the subcutaneous tissues of the scrotum. The lesions first formed were simple ulcers surrounded by a thin, parchment-like zone of induration in which spirochetes were present (Fig. 33, 35 days after inoculation). On the left, the zone of induration increased slowly and a characteristic skin chancre was produced (Fig. 34, 83 days after inoculation). Meanwhile, the skin lesion on the right had regressed, and a small nodule had developed in the subcutaneous tissues (Fig. 34). Then followed a period during which the lesion on the left extended into the subcutaneous tissues, while the induration in the skin diminished and the ulcer healed (Figs. 35 and 36, 139 and 188 days after inoculation).

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[2] In this connection, it may not be out of place to call attention to the fact that in all syphilitic infections, the presence of a toxin as an exciting factor distinct from the organisms must be kept in mind. We have avoided introducing this element into the discussions, since we are not prepared, as yet, to make any definite statement upon this point. It seems not unlikely, however, that certain features of the syphilitic reaction may find their explanation in the action of such a toxin, and this applies to reactions of the type just described.

A second case of even less characteristic chancre formation is shown in Figs. 37 to 40. The initial skin lesion in this animal was no more than a thickened patch, the surface of which was smooth and glistening with small erosions here and there; some of these were covered by scales, while others were of a weeping character. These lesions underwent many transformations (Figs. 37 to 40), but never developed beyond the point shown in Fig. 37 which represents the condition existing 36 days after inoculation. Here again, the granulomatous lesions which ultimately developed were subcutaneous in origin and were of very slow and irregular growth (Figs. 38, 39, and 40; 92, 106, and 127 days after inoculation).

Diffuse Scrotal Reactions and Transformations of Diffuse Lesions.

As we have already pointed out, there is a tendency on the part of *pallidum* infections to spread beyond the point of inoculation, and not infrequently this local extension of the infection gives rise to lesions of a more diffuse character than those which have been described. No sharp line of distinction could be drawn between these two groups of lesions; it appeared that one was but a step removed from the other, and diffuse and circumscribed processes were frequently coexistent, or one type of lesion might be transformed into the other, instances of which have already been noted.

As a connecting link between these two forms of scrotal reaction, attention may be called to the lesions shown in Figs. 41 to 44. The photograph reproduced in Fig. 41 was taken 33 days after inoculation. In this animal, there was a moderate but fairly well defined thickening in the skin about the incisions as well as about the implants, and the characteristic feature of the lesions was the spreading necrosis in the skin over the implants and the formation of loosely attached scales, or exfoliation, over all affected parts of the skin, which is shown very well in the left scrotum (Fig. 41).

The second photograph (Fig. 42) shows a later stage of a similar lesion. In this animal, there are two things to be noted, first the puckered scar-like areas on both sides, and second the peculiar appearance of the skin about these areas. The skin was in general of a parchment-like character, very smooth and translucent with grayish yellow or yellowish brown scales or crusts distributed over its surface. Needless to say, these portions of the scrotum contained spirochetes in abundance.

The other two figures on this plate (Figs. 43 and 44) illustrate a somewhat different manifestation of the syphilitic infection. In Fig. 43, there is shown a rather irregular and slightly nodular thickening in the right scrotum with a scurfy condition of the skin over portions of the lesion; on the left, there is a puckered ulcer with moderate thickening of the surrounding skin. These were the lesions as

they appeared 76 days after inoculation. Subsequently the thickening or induration in the skin became diminished, but instead of healing, the lesions spread in an irregular way, producing a peculiar serpiginous necrosis and ulceration over a considerable part of the scrotum (Fig. 44, 118 days after inoculation). There was no suggestion of an acute inflammatory process associated with these changes, and spirochetes were present in fluid drawn from about the lesions.

The next series of photographs (Figs. 45 to 48) shows successive stages in the transformation of a diffuse scrotal infection which reverses somewhat the sequence of events illustrated in the preceding series. The reaction began here as a diffuse infiltration with exfoliation of the epithelial covering of the scrotum (Fig. 45, 53 days after inoculation). After a time, the scaling, or exfoliation, ceased, and the skin became very smooth and translucent. At the same time, the skin became diffusely indurated, and circumscribed nodules appeared in the subcutaneous tissues on both sides. These nodules grew rather slowly, and on the 151st day after inoculation, the condition presented was that shown in Fig. 46. The subcutaneous nodule in the left scrotum was later excised, but the infection in the scrotum persisted in spite of the operation. The nodule on the right continued to grow, and as it developed, the infiltration in the skin diminished. There was, however, a slight recurrence of the exfoliative reaction (Figs. 47 and 48, 165 and 172 days after inoculation).

No attempt can be made to indicate the diversity of conditions to which the specific reaction in the scrotum may lead. By comparing and following the various phases and stages of the reaction, it will be seen that the elements in these reactions are the same in all cases; all show a vascular reaction, a certain amount of exudation, infiltration, and proliferation together with secondary necrosis and ulceration or exfoliation where the necrosis is superficial. The lesions we regard as but an expression of the operation of these factors in the reaction and the balance obtaining in a given case at a given time. As the response to the specific infection varies in different animals or even in the same animal from time to time, so do the lesions, and the possibilities of such variations are almost infinite. The consid ration of this phase of the local infection will be taken up in the second part of this paper.

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EXPLANATION OF PLATES.

The first four illustrations are drawings made from the living animal and are based upon photographs taken at the time. The remaining illustrations are reproductions of untouched photographs. All objects are represented at their natural size. The statements of time refer in all cases to the time after inoculation unless otherwise stated.

PLATE 72.

FIGS. 1 to 4. Successive stages of the reaction to infection in the scrotum following inoculation with a virus emulsion.

FIG. 1. 2 weeks. Localized swelling in the scrotum with vascularization of the area. An unusually characteristic reaction.

FIG. 2. 23 days. Early induration with congestion and petechial hemorrhage from the vessels in the center of the lesions.

FIG. 3. 28 days. Anemic necrosis and exfoliation.

FIG. 4. 41 days. Circumscribed and indurated chancres with marked central necrosis and ulceration.

PLATE 73.

FIGS. 5 to 8. Development of scrotal chancres from focal infiltrations following inoculation with a virus emulsion.

FIG. 5. 1 week. Minute focus of induration in the right scrotum and diffuse infiltration with vascularization in the left.

FIG. 6. 19 days. Circumscribed areas of infiltration and induration. The skin over the center is becoming thin and translucent; slight congestion and hemorrhage on the right.

FIG. 7. 28 days. Surface necrosis and exfoliation.

FIG. 8. 43 days. Chancres with well developed collars of induration and marked central necrosis and ulceration.

PLATE 74.

FIGS. 9 to 12. An intense reaction to infection with the development of circumscribed indurated chancres and subsequent extension of the lesions following implantation in the scrotum.

FIG. 9. 10 days. The early syphilitic reaction following inoculation by implantation. There are diffuse edema and congestion of the scrotum and the implants are surrounded by a narrow zone of induration, while the skin over their center has been converted into hard dry crusts.

FIG. 10. 13 days. The early chancre. Note especially the well marked collars of induration and the tendency to exfoliation in the right scrotum.

FIG. 11. 22 days. The diffuse reaction has persisted and the edema has given place to infiltration and thickening of the scrotum.

FIG. 12. 36 days. Large indurated chancres. Diffuse extension of the reaction with widespread necrosis and ulceration of the scrotum.

PLATE 75.

FIGS. 13 to 16. A usual case of scrotal reaction showing cyclic changes in the development of the lesions. Scrotal implantation.

FIG. 13. 14 days. Typical circumscribed lesions with depressed ulcers; edema and congestion of the scrotum.

FIG. 14. 21 days. The lesions have decreased in size; edema and congestion have subsided.

FIG. 15. 28 days. Renewed growth of the chancres and reappearance of scrotal edema.

FIG. 16. 57 days. Large indurated chancres with well marked central necrosis and the formation of ulcers.

PLATE 76.

FIGS. 17 to 20. Deep seated scrotal infection, characterized by slow growth of the lesions and late involvement of the skin. Implantation.

FIG. 17. 34 days. An early stage of the reaction. Skin but slightly involved.

FIG. 18. 41 days. Involvement of the skin with obliteration of papillæ and beginning skin necrosis.

FIG. 19. 44 days. A very active advance of the process indicated in Fig. 18.

FIG. 20. 52 days. The reaction is still quite active. The lesions are extending laterally and the central necrosis is increasing.

PLATE 77.

FIGS. 21 to 26. Multiple foci of primary reaction. Multiple chancres Implantation.

FIG. 21. 14 days. A well marked accessory focus of reaction at the point of incision of the scrotum on the right.

FIG. 22. 36 days. Overgrowth of the accessory chancre by the main focus of reaction.

FIG. 23. 29 days. Quadruple foci of reaction. The accessory lesion on the right is already regressing.

FIG. 24. 29 days. Triple and double foci of reaction.

FIG. 25. 49 days. Subsequent development of chancres from four of the five initial foci of reaction in Fig. 24.

FIG. 26. 47 days. Quadruple chancres. An unusual case of equal reaction about all of four centers of reaction.

PLATE 78.

FIGS. 27 to 32. Variations and irregularities of the specific reaction. Edema, congestion, and acute inflammatory reactions.

FIG. 27. 16 days. Early circumscribed chancres with diffuse congestion and marked edema of the scrotum. Spirochetes recovered from the point indicated by the arrow on the right.

FIG. 28. 25 days. Focal lesions with acute edema and congestion of the scrotum.

FIG. 29. 39 days. Marked growth of the focal lesions following the exudative reaction in the scrotum.

FIG. 30. 102 days. Sudden development of edema in the scrotum which was the seat of an old indurated lesion. (Compare left.)

FIG. 31. 11 days. An intense granulomatous reaction associated with edema and marked congestion of the scrotum, the exact nature of which is uncertain (see text).

FIG. 32. 50 days. The same animal as in Fig. 31, showing the development of typical indurated chancres following partial regression of the previous lesions.

PLATE 79.

FIGS. 33 to 36. Successive stages of an atypical granulomatous reaction.

FIG. 33. 35 days. Simple ulcers of the scrotum with slight induration about their margins and base.

FIG. 34. 83 days. Slow but characteristic development of the lesion in the left scrotum. Regression of the lesion on the right with the formation of a small subcutaneous nodule underneath the original lesion.

FIG. 35. 139 days. Continued growth of the lesion on the left while that on the right has diminished in size.

FIG. 36. 188 days. Complete healing of the original skin lesions with the development of active subcutaneous nodules on both sides.

PLATE 80.

FIGS. 37 to 40. Successive stages of an irregular scrotal reaction.

FIG. 37. 36 days. Patches of superficial induration in the skin with necrosis and exfoliation.

FIG. 38. 92 days. The patch of induration on the left is still present, while that on the right has almost completely resolved. Subcutaneous nodules forming on both sides.

FIG. 39. 106 days. On the right, there is a small patch of glassy induration in the skin and beneath this a small indurated nodule surrounded by a mass of diffusely thickened tissue. On the left, the subcutaneous nodule is developing rapidly.

FIG. 40. 127 days. Active development of both nodules with extension to the skin. Note the recurrence of the indurated patch in the right scrotum.

PLATE 81.

FIGS. 41 to 44. Diffuse syphilitic processes following scrotal inoculation.

FIG. 41. 33 days. Slight granulomatous reactions about both the implants and the points of incision in the scrotum. The characteristic feature of the reaction is the spreading necrosis and exfoliation over the areas involved.

FIG. 42. 105 days. Diffuse infiltration about the site of inoculation with superficial necrosis and exfoliation. No typical chancre was ever formed in this animal.

FIG. 43. 76 days. A group of irregular and slightly nodular lesions in the right scrotum with surface necrosis and slight exfoliation. On the left, there is a diffuse infiltration of the scrotum with necrosis over the central area.

FIG. 44. 118 days. Same animal. Spreading serpiginous necrosis involving the areas of infiltration.

PLATE 82.

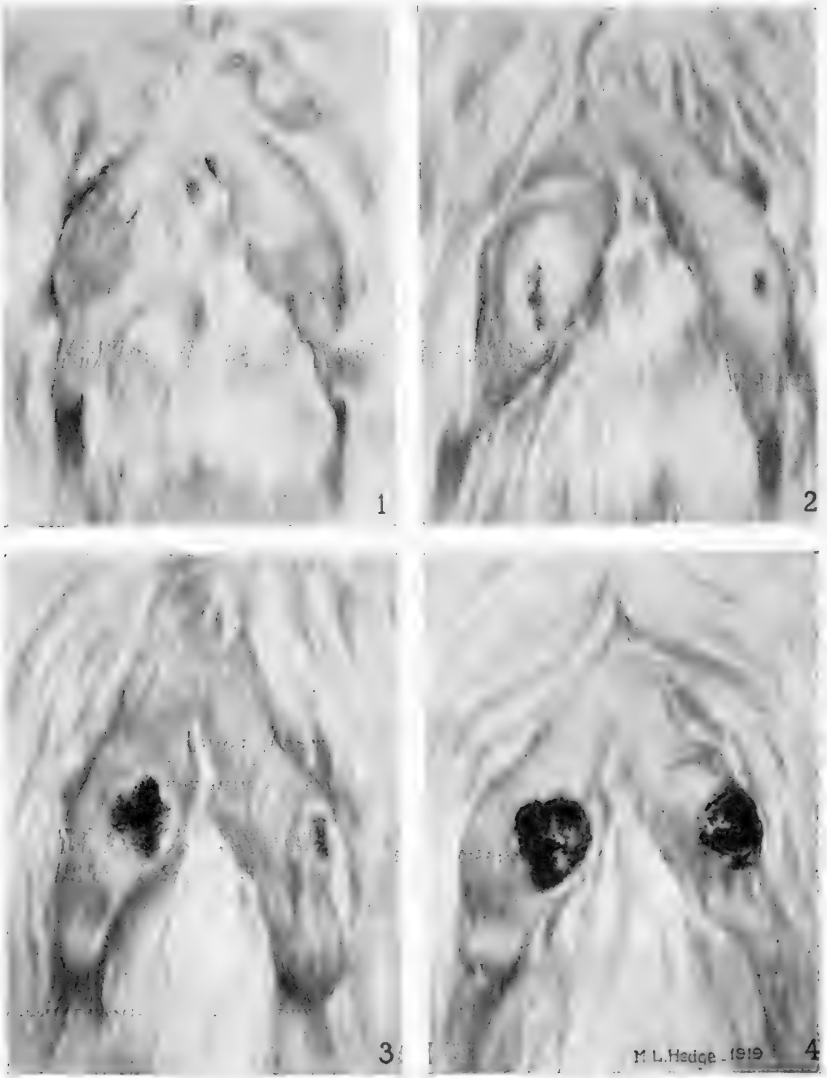
FIGS. 45 to 48. Successive stages in the transformation of diffuse scrotal lesions.

FIG. 45. 53 days. Diffuse infiltration of the scrotum, slight necrosis, and well marked exfoliation of surface epithelium—the initial lesion.

FIG. 46. 151 days. Marked diffuse infiltration of the skin with obliteration of the papillæ; large granulomatous nodules developing in the subcutaneous tissues.

FIG. 47. 165 days. Subcutaneous nodule removed on the left; that on the right still increasing, while the infiltration of the scrotum is slightly diminished.

FIG. 48. 172 days. The operative wound is practically healed; active infection still persists in the skin on the left; the nodule in the subcutaneous tissues is increasing, but the infiltration of the skin has almost disappeared; there is slight desquamation of surface epithelium.



(Brown and Pearce: Experimental syphilis in the rabbit. II.)

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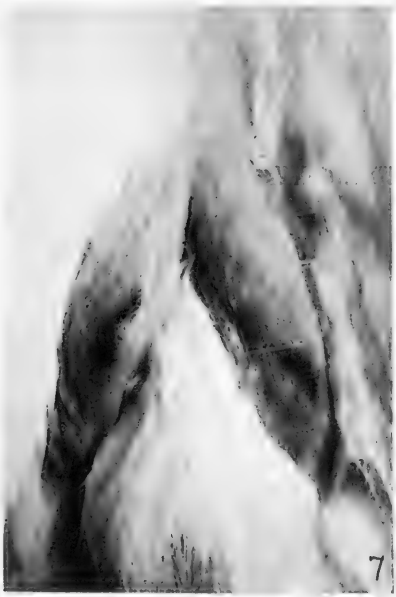

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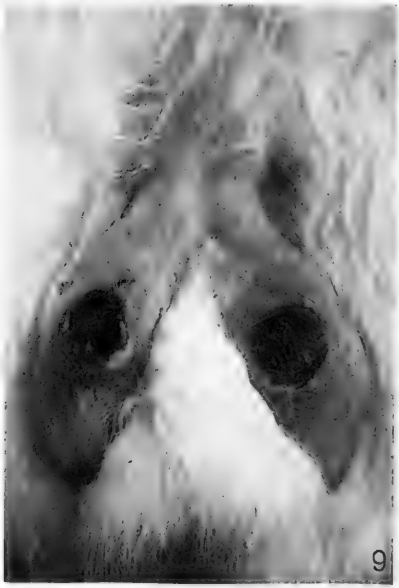

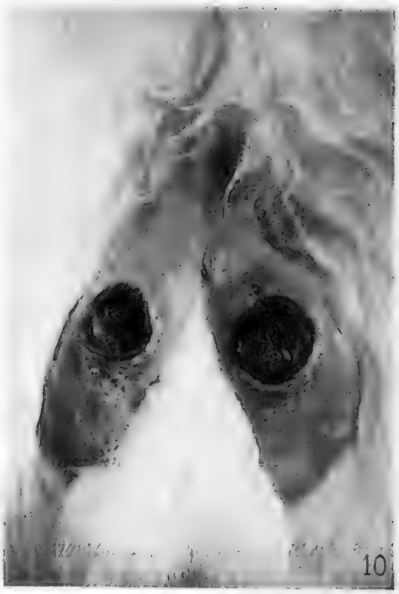

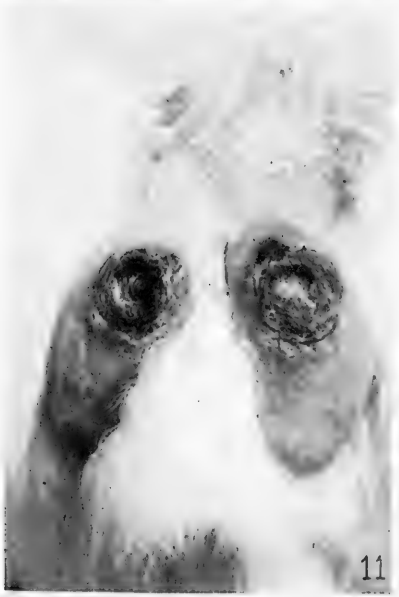

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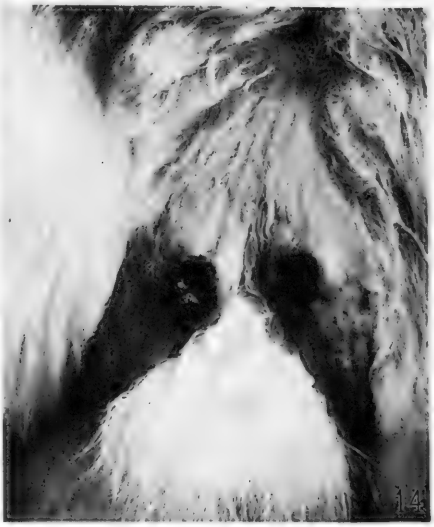

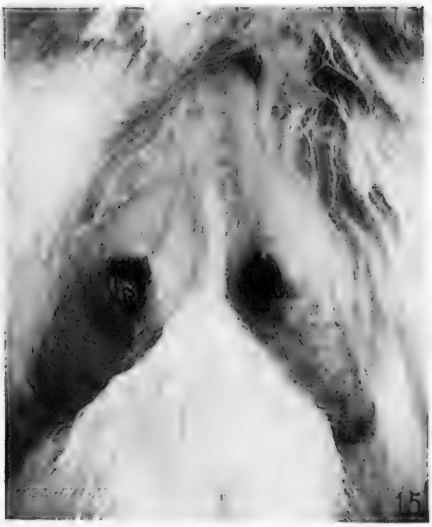

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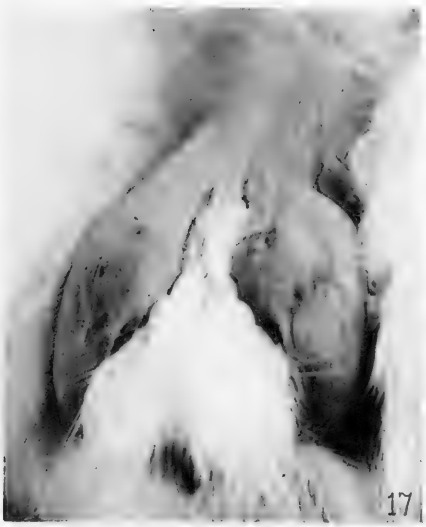

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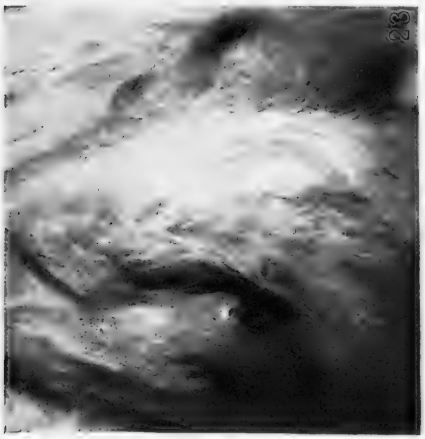

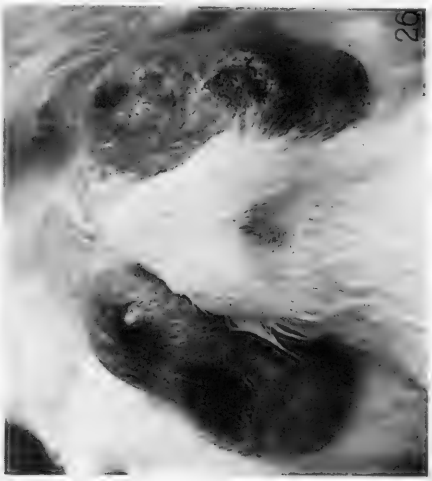

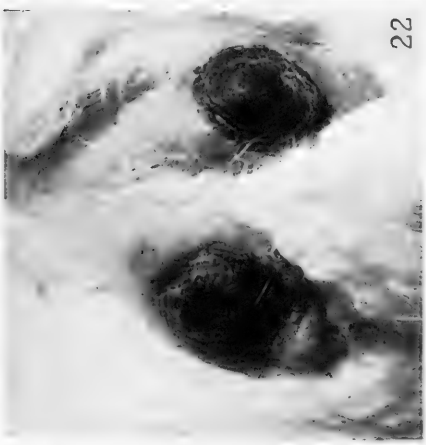

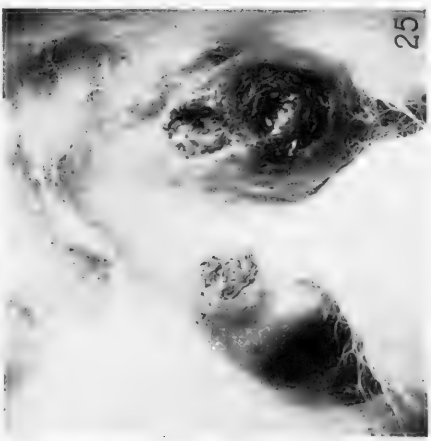

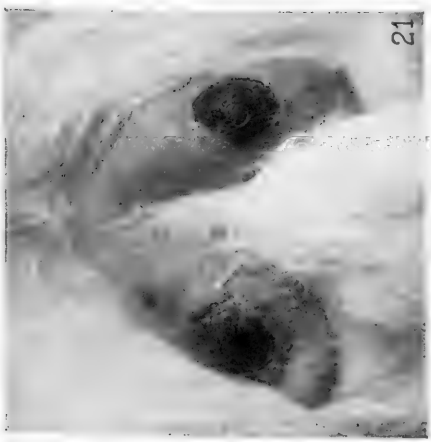

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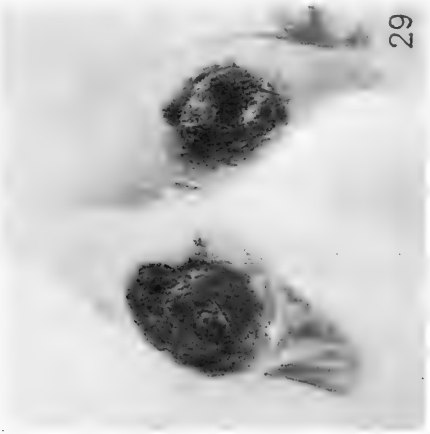

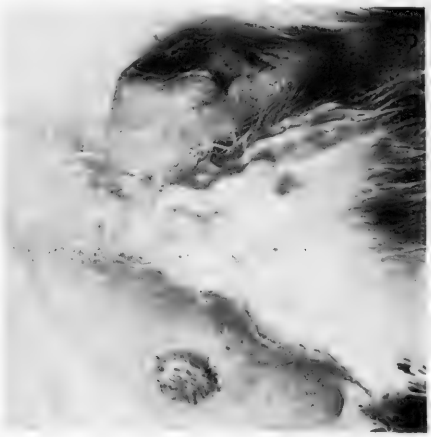

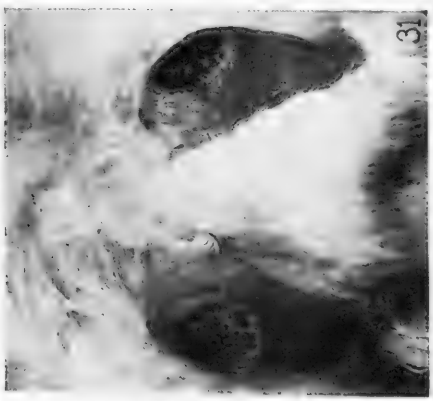

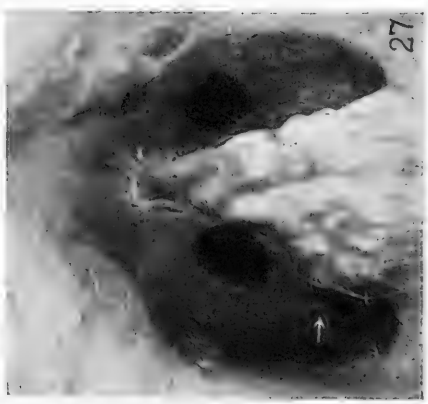

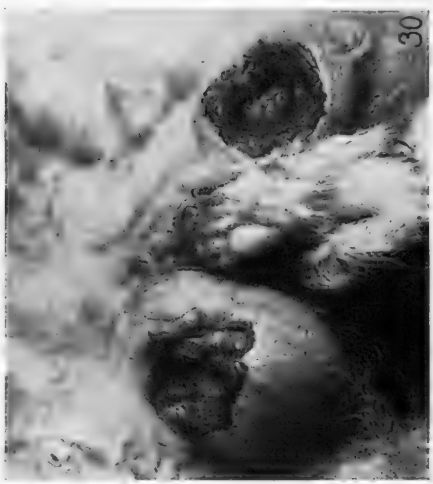

(Brown and Pearce: Experimental syphilis in the rabbit. II.)

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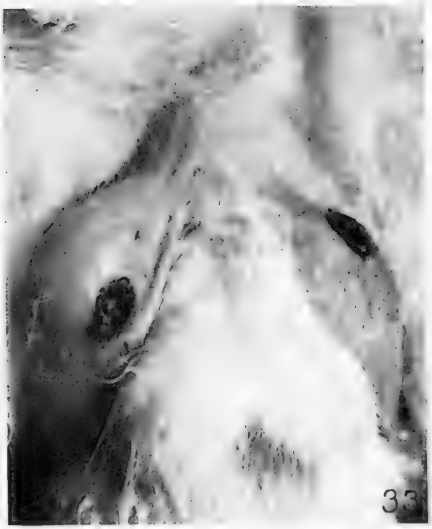

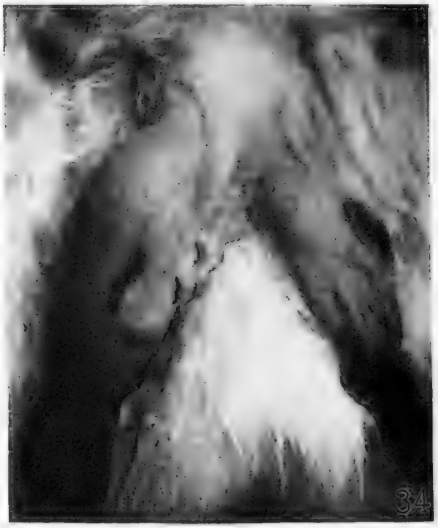

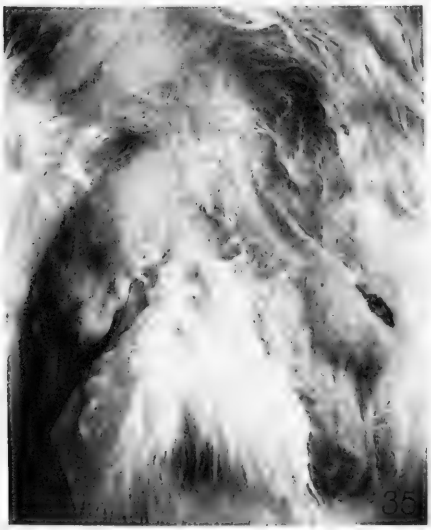

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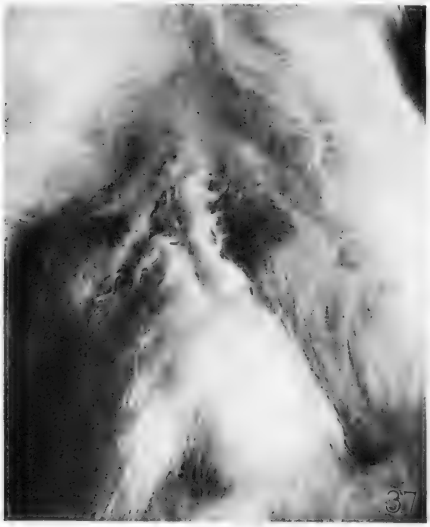

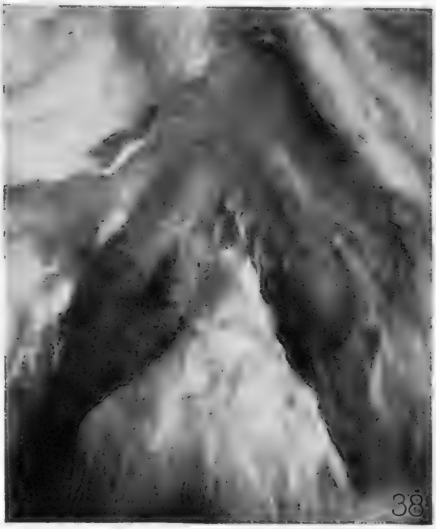

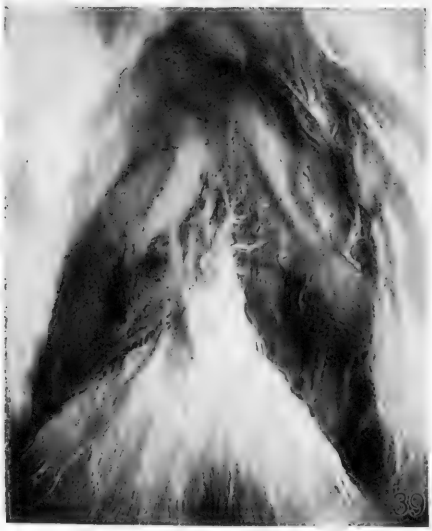

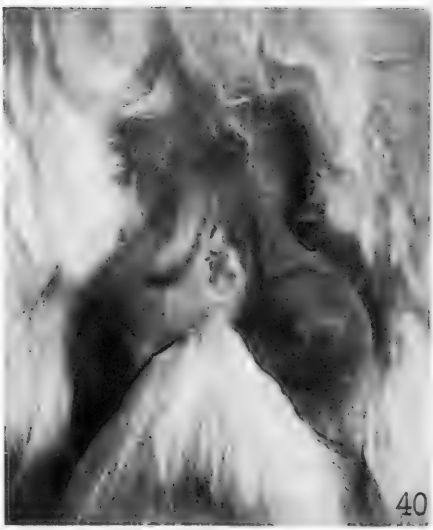

(Brown and Pearce: Experimental syphilis in the rabbit. II.)

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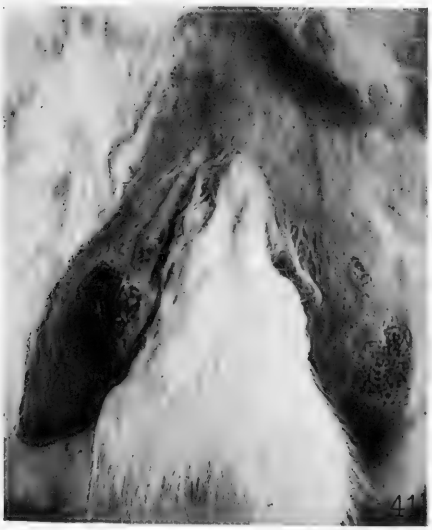

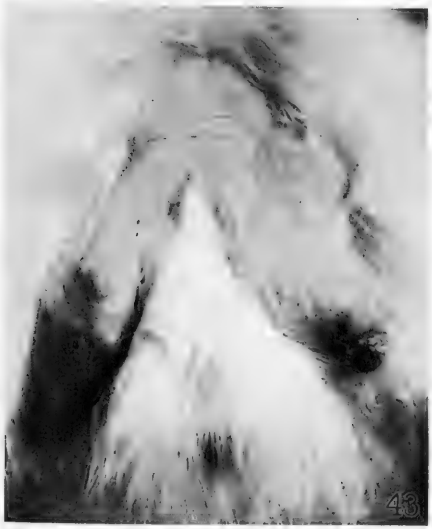

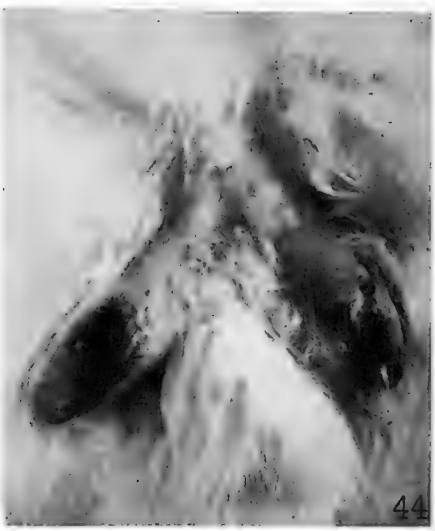

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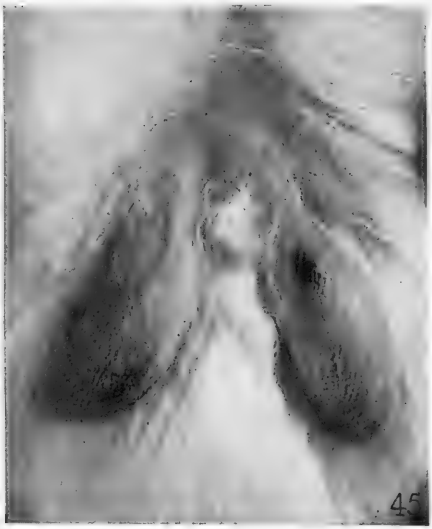

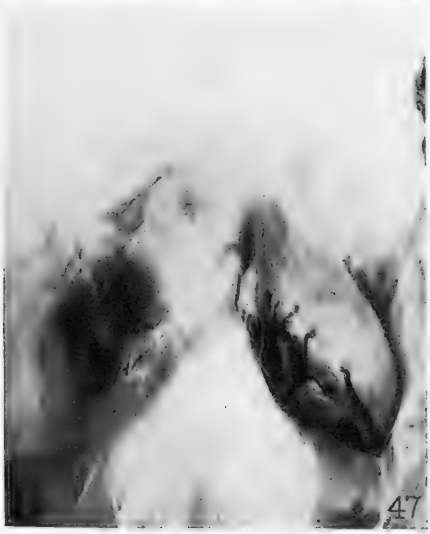

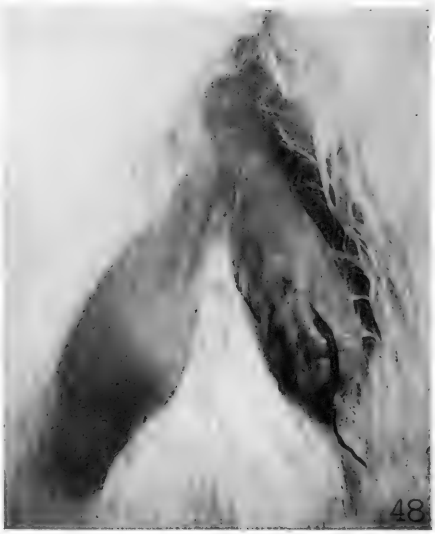

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EXPERIMENTAL SYPHILIS IN THE RABBIT.

II. Primary Infection in the Scrotum.

Part 2. Scrotal Lesions and the Character of the Scrotal Infection.

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Plates 83 to 89.

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In Part 1 of this series, the subject of scrotal syphilis in the rabbit was considered from the standpoint of the local reaction to infection. The various elements in this reaction were noted, and the process was traced from the time of inoculation to the development of characteristic skin lesions. Especial attention was called to the multiform character of these reactions and the influence of various elements in the reaction upon the character of the lesion produced, and while cause and effect were not entirely dissociated, the main emphasis was placed upon the process, and no detailed description of the resulting lesions was attempted. This phase of the subject is, however, of great importance in itself, and a more comprehensive view of the scrotal lesions is essential to a correct understanding and use of the experimental infection.

Primary Lesions of the Scrotum.

A classification of scrotal lesions which would be acceptable from all standpoints is impossible, but in order to simplify the description of these lesions, they may be separated into several general groups based partly upon fundamental differences in growth tendencies and partly upon structural differences in the lesions themselves, neither of which represent sharply defined or entirely fixed characteristics.

The first division to be made is one which has already been suggested, that of circumscribed and diffuse lesions. The circumscribed chancres include the lesions in which growth takes place from a central focus and tends to be more or less equal in all directions, leading to the development of raised or elevated masses of a circumscribed and indurated character. The diffuse chancres, on the other hand, include lesions which tend to spread laterally and assume a more or less flattened condition. In our experience, chancres of the first group were by far the more common of the two, and since they are more analogous to the classical primary lesions seen in man, they might also be spoken of as typical chancres in contradistinction to the less well defined lesions of a diffuse character.

The variety of primary lesions and the differences which exist between extreme examples of these two groups of lesions are so great that without the intervening links they could hardly be recognized as results of one and the same pathological process. Considerable care has been devoted, therefore, to the mere matter of selection and arrangement of illustrations with a view to giving as accurate an impression of scrotal chancres as possible, both as regards the character of the lesions and the relationship existing between lesions of different types.

Circumscribed or Typical Scrotal Chancres.

The circumscribed, indurated lesions of the scrotum studied by us were lesions which as a class tended to grow to a very large size—much larger than the primary lesions commonly seen in man. These lesions differed according as they arose from one part of the scrotum or another and according to the mode and rate of their development. Based upon these differences, the typical chancres of the scrotum might be divided into two main groups, the nodular and the lenticular or discoid chancres.

Nodular Chancres.—The nodular chancres comprise a large group of lesions whose chief characteristics were an irregular spherical form, a relatively small and sharply circumscribed area of superficial necrosis and ulceration, or the absence of any ulceration, and extreme induration. This general class of chancres may be separated into two

divisions, the ulcerated and the unulcerated chancres, characteristic examples of which are given in Figs. 1 to 6 and 7 to 12.

The nodular chancres appeared to develop from the subpapillary layers of the skin, the subcutaneous tissues, or the outer surface of the tunica vaginalis. They began their existence as more or less spherical masses of induration situated in the deeper portions of the scrotum. The growth of the lesions was comparatively rapid, while necrosis took place more slowly and was of relatively slight extent, at least during the period of active development of the lesion. As the overlying skin became involved, therefore, the area of necrosis was small and a sharply circumscribed depressed ulcer was formed, the margins of which were rounded and intensely indurated (Figs. 1 and 2). These lesions were of a dense fibrous structure, and the small area of central necrosis was usually surrounded by a thick wall of well vascularized living tissue.

The chief variations among the chancres of this class appeared to have their origin in the balance existing between the phenomena of growth and necrosis. The typical condition was that shown in Fig. 1. In a second group of cases (Figs. 2 and 3), growth and necrosis were more nearly balanced and a more extensive destruction of the central portion of the lesion took place. This condition, however, appeared to be referable to a corresponding variation in the structure of the surrounding tissue which in these cases was unusually dense or of almost ivory-like hardness. This condition is suggested in Fig. 2.

The chancres reproduced in Figs. 4 and 5 illustrate another modification commonly seen in chancres of this group occurring either during the period of active growth or as a terminal alteration. The condition was no more than a spreading necrosis which in actively growing lesions produced a unilateral flattening due to cessation of growth in the direction of the skin surface. This alteration was especially noticeable with large and rapidly growing lesions such as those in Figs. 4 and 5. It was a common occurrence, however, with all classes of chancres after active growth had slackened or ceased. In these cases, extension of the necrosis due to a continuation of secondary alterations in the granulomatous tissue resulted in a gradual destruction of the blood vessels and shutting off of the blood supply to the chancre.

This secondary necrosis took place in one of two ways which may be suggested by a comparison of the right and left chancres in Figs. 5 and 6. With large lesions showing a unilateral flattening with surface necrosis, there was a tendency for the spreading necrosis to surround the lesion from the outside (Fig. 5), while in lesions with central necrosis and depressed ulcers, the extension of the necrosis took place radially. This latter type of change, as illustrated in Fig. 6, was, of course, not confined to terminal alterations in chancres but occasionally was seen as an early feature of the lesion and is entirely analogous to the condition shown in Fig. 3.

The second division of nodular chancres differed from the first chiefly in respect to necrosis and ulceration. The two groups of lesions had a common origin and a common structure; in one group, surface necrosis and ulceration of moderate degree were characteristic features of the lesions, while in the other, these secondary alterations were of very limited extent or entirely absent. The photographs reproduced in Figs. 7 to 12 have been arranged to show successive gradations in the tendency to skin involvement and ulceration.

It may be of interest to note that all the chancres used for illustration in Figs. 1 to 12 were produced by a common organism—the Zinsser-Hopkins strain of *Treponema pallidum*. Formerly, lesions of the second type were the more common of the two, while at present the order of frequency is reversed.

Lenticular and Discoid Chancres.—The lenticular and discoid chancres which form the second main division of typical scrotal chancres differed from the first or nodular chancres in being lesions of a more flattened character, in the occurrence of a more widespread necrosis, and in possessing a lesser degree of induration as a rule. Typical examples of lesions of this class are given in Figs. 13 to 24.

The lenticular and discoid chancres appeared to arise mainly from the papillary layers of the skin, and the chief direction of their growth was in a plane parallel with the skin surface. Some of these chancres showed the thicker center and sloping edges of the lenticular lesion, but more commonly the edges were elevated fully as much or even more than the center, giving to the lesions a discoid rather than a lenticular form.

The growth of these chancres was extremely rapid, as a rule, and was associated with widespread skin involvement. Early in the course of their development, the skin over the central portion of the lesion became necrotic, and the extension of this zone of necrosis tended to keep pace with the growth of the lesion, spreading over the surface of the lesion as well as through its deeper parts. In some instances, the necrotic area softened and sloughed away with the formation of a true ulcer, but in others, the necrotic tissue remained firmly attached to the underlying structures, forming a thick imbricated crust (see figures).

Outside the zone of necrosis, the skin covering these chancres was markedly infiltrated and presented an unusually smooth and translucent appearance—a condition which not infrequently extended entirely to the outer edges of the lesions.

These chancres were as a class less indurated than the nodular chancres. In exceptional instances, however, they showed the same dense, fibrous structure and intense induration as nodular chancres (see Fig. 27), but more commonly they were of a mucoid or cellular character, and while these lesions were indurated, their induration was of an elastic quality, giving the impression of tension rather than of hardness.

Rapid growth and widespread necrosis were such striking characteristics of this group of chancres that two sets of illustrations are given to show the extremes to which these processes may go. Figs. 19 to 21 represent chancres produced by inoculation with a virus emulsion. The original lesions in this animal were excised 20 days after inoculation. There was a prompt recurrence, however, and Fig. 19 shows the lesions present 45 days after inoculation, or 25 days after excision of the lesions. From this point on the evolution of these chancres was extremely rapid. Within 20 days, they increased to the size shown in Fig. 20; during the next 3 weeks, growth continued at a somewhat slower rate with a deepening and extension of the necrosis (Fig. 21). The chancre on the left appears here much smaller than that on the right but was in reality almost as large. The appearance is due to foreshortening as only one chancre could be brought squarely in front of the camera.

The other three figures (Figs. 22 to 24) are from chancres produced by implantation. They show an equally rapid growth with the production of lesions which in this case almost lapped the testicles. The necrosis in these chancres was of the type of a dry gangrene spreading diffusely over the surface without a slough and forming heavy crusts composed of concentric layers and rings of ne-

crotic tissue. The figures represent periods of 39, 46, and 60 days respectively after inoculation.

The entire group of lenticular and discoid chancres thus far presented from Figs. 13 to 24 were produced by the Nichols strain of *Treponema pallidum* and show a certain degree of uniformity in type. Some of the more important variations of this class of chancres are indicated in Figs. 25 to 30.

The first two chancres of this group (Figs. 25 and 26) are of a decidedly mucoid character and the skin over the lesions is quite smooth and translucent. One of these chancres is flat while the other shows a tendency towards a more nodular form. In contrast to these lesions which again were products of the Nichols' organism, the two chancres in Figs. 27 and 28 present a decidedly different appearance. They were older lesions but represented a state of development comparable to that of the other two lesions. These chancres were of a dense fibrous character and were intensely indurated. They were produced by the Zinsser-Hopkins strain of *Treponema pallidum*.

The two remaining photographs of this group (Figs. 29 and 30) are given to illustrate chancres of an indolent type or ones which are less vigorous than most of those previously shown. The first of these chancres showed a normal rate of growth but an irregular growth; the lesions were only moderately indurated and there was but slight skin involvement apart from the area of necrosis. The second set of chancres (Fig. 30) showed a very slow and irregular development, with an undermining necrosis. The skin about the lesions was relaxed and there was the merest shell of living and growing tissue. Lesions such as those in Figs. 29 and 30 are more or less constantly at the border-line of regression and their growth may be interrupted by the slightest of causes.

The chancres thus far described will serve to give a fair impression of the principal chancre types and of the scrotal chancre at its highest state of development. The modifications of these types were so numerous that it would be futile to attempt to describe such a series of lesions. As we pass from these more typical chancres, we come to a group of lesions showing a progressive loss of the characteristics by which we are accustomed to identify primary skin lesions and eventually to lesions which are quite atypical in character. The photographs reproduced in Figs. 31 to 36 are intended to indicate this transition from the typical circumscribed chancres to lesions of a more diffuse and less typical character.

The first three photographs (Figs. 31 to 33) show lesions with all the characteristics of active skin lesions. The chancres in Fig. 31 were virtually thickened plaques with wide shallow ulcers, but the narrow margins as well as the base were markedly indurated. The chancres in Fig. 32 were somewhat less vigorous;

in the right scrotum there was an irregular indurated nodule with a depressed ulcer, and the flattened plaque on the left showed the merest shell of induration. Fig. 33 again shows a nodule or a lenticular thickening in the skin of the right scrotum which fades into the surrounding tissue. On the left, however, there is an extremely small but perfectly characteristic chancre. These photographs represent what was practically the highest point reached in the development of these lesions.

The next three illustrations of this group represent lesions which are decidedly less characteristic. The small ulcer in the right scrotum of the animal shown in Fig. 34 has a definite collar of induration, but on the left, there is little more than a minute sharply defined ulcer with a suggestion of a diffuse thickening in the scrotum. The next lesions (Fig. 35) consisted of irregular areas of thickening with even more irregular areas of ulceration. These lesions represented the height of the local reaction in this animal, and it may be of interest to note that generalized lesions appeared elsewhere just at this time (58 days after inoculation). The final photograph of the series (Fig. 36) shows small nodular lesions in the scrotum of both testicles which persisted for months with almost no change from the condition here shown.

Atypical and Diffuse Lesions of the Scrotum.

The lesions classed as atypical and diffuse include conditions varying from chronic ulcers and nodular thickenings in the scrotum to various inflammatory processes of an ill defined character. As a class, these lesions persist for a long time, and while there is usually no considerable difficulty in demonstrating the specific nature of the lesions, they are subject to frequent transformations which make it very difficult to say whether they should be regarded as primary or as secondary manifestations of infection, or where the line of separation between the two should be placed. At all events, the lesions are the same in either case, and since one group cannot be clearly differentiated from the other, they must be considered here without reference to their primary or secondary character.

Several groups of lesions belonging to this class have already been described and illustrated in Part 1 of this paper. These descriptions of atypical and diffuse scrotal lesions may be supplemented by further examples of lesions of a somewhat different type.

The photographs reproduced in Figs. 37 and 38 represent forms of primary scrotal lesions which were very commonly seen and frequently were the starting points for lesions such as those in Figs. 39 to 42. The first of these photographs

(Fig. 37) was taken 43 days after inoculation and shows a small indurated nodule with an apical ulcer in the right scrotum and an area of diffuse infiltration in the left. The skin in this area showed a loss of papillæ and increased translucency together with the formation of yellowish white scales over its central portion. The second photograph (Fig. 38) shows changes of an analogous character but somewhat more pronounced. The infiltration of the scrotum was more marked on both sides, and on the right there were definite areas of superficial necrosis and exfoliation. This photograph was taken 77 days after inoculation, and just at this time, patches closely resembling those in the right scrotum made their appearance upon the skin at the base of the ears. While neither set of the scrotal lesions shown (Figs. 37 and 38) might conform to the usual conception of a chancre, they were nevertheless the primary lesions of these animals and are just as characteristic of *pallidum* infection in the rabbit as any of the lesions previously described.

Fig. 39 represents another form of scrotal syphilide which occurred either as the starting point of an infection (primary lesion) or as a transformation of other types of lesions. This animal was kept under observation for 29 months after inoculation and never developed lesions more analogous to the ordinary chancre than those shown in Fig. 39, the photograph of which was taken 136 days after inoculation. Altogether, these lesions persisted in essentially the form represented for about 18 months and this animal showed a most marked generalized infection.

The lesions shown in Fig. 40 (190 days after inoculation) are a somewhat different form of the same process as that in Fig. 39. In this animal, there was a diffuse thickening of the scrotum of both testicles, most marked in the dependent portions. On both sides, there was a curved ridge (shown in the photograph only on the right) extending downward and spreading out into a flattened head at the lower end of the scrotum. This ridge and portions of its terminal expansion were profusely covered with scales and thin crusts with erosions here and there.

Finally, there are two illustrations (Figs. 41 and 42) of lesions which in their later transformations tended to revert to a form more like an ordinary chancre. The initial lesions of the animal shown in Fig. 41 were circumscribed nodular lesions with marked congestion and edema of the scrotum. These lesions were of short duration, and by the end of the 5th week after inoculation, they had almost disappeared, leaving a diffuse thickening of the scrotum analogous to that shown in Fig. 37 or 38. 94 days after inoculation (Fig. 41) there was involvement of both testicles (orchitis), diffuse thickening of the scrotum with the formation of fine bran-like scales (shown fairly well on the left), and indolent ulcers on both sides with thickening but no induration about them. These lesions persisted with some further transformations of an equally atypical character up to the time the animal was discarded 11 months after inoculation.

The last photograph of the series (Fig. 42) represents the lesions present in the scrotum of a rabbit 316 days after inoculation. The infection in this animal

began with the formation of lesions almost identical with those shown in Fig. 35 and remained a diffuse infiltrative and exfoliative type of process for upwards of 9 months. It was not until during the 10th month after inoculation that circumscribed lesions of the character shown in Fig. 42 began to appear. On the right, the lesions became confined almost entirely to the nodular mass at the lower end of the scrotum, but at least two-thirds of the left scrotum was the seat of a diffuse infiltration and at times showed a tendency to the formation of scattered exfoliative lesions. (Note the dark colored spots on the scrotum which represent areas of recent exfoliation.) In the midst of this diffuse process, the chancre-like lesion shown in the photograph was formed.

From the standpoint of the human infection, this group of atypical and diffuse lesions of the scrotum is of the utmost importance. They are in many instances lesions which at first glance do not suggest syphilis or at least primary syphilis. They have doubtless been noted by many observers, but very little attention has been paid to them, possibly for the reason that they were interpreted as evidences of a low grade or slight infection which is not necessarily the case.

We have had many rabbits with lesions of this type, a large proportion of which came from the Zinsser-Hopkins strain of *Treponema pallidum*. These animals were of no particular use for therapeutic experiments, and consequently only a comparatively small number of them was kept under observation for any considerable period of time. Those kept were held partly with the hope that they might eventually develop lesions which could be used and partly for the purpose of studying this particular class of infections. It was in this way that we learned what we have about them.

As a class, these atypical and diffuse lesions persisted fully as long or longer than any other class of primary lesions; they contained actively motile spirochetes in abundance, and these organisms were highly virulent which has been demonstrated in two ways, first by the fact that rabbits in which these lesions occurred were frequently the subjects of severe generalized infections, and next that organisms taken from such lesions also produced high grade infections when inoculated into other animals.

General Course of the Local Infection.

Judged upon the basis of the changes which took place in the lesions or by the reaction in the scrotum, the course of the scrotal infection appeared to be fundamentally the same as that in the testicle, modified undoubtedly by differences in the character of the tissue within which or from which the lesions developed. In the scrotum, as in the testicle, there were evidences of periodic changes, but the specific reaction in the scrotum was more stable than that in the testicle and less subject to marked or rapid changes in one direction or another. While one could distinguish between lesions or processes which were diffuse and those which were circumscribed, it was more difficult to draw a sharp line of demarcation between processes which were acute or exudative and those which were proliferative in character.

In general, the initial reaction in the scrotum usually progressed without interruption until a well defined lesion had been produced or for some 2 or 3 weeks at the least. The reaction then assumed an irregular character with periods of growth interrupted by longer or shorter intervals during which little or no change could be detected in the lesions, or during which the lesions appeared to regress. As long as the lesion was merely quiescent or inactive, it retained its appearance unaltered, but when regression set in, the skin about the lesion became relaxed and wrinkled, the induration softened, or the lesion diminished in size. At times, the entire lesion was affected by these changes, while at others, only certain parts of the lesion were affected, or, as in the case of the testicular infection, one portion of the lesion might be actively developing while another was rapidly regressing.

The time element in these changes was most uncertain—some lesions lay dormant over long periods of time (several months) and then grew actively, while others grew steadily for a long time before showing any sign of cessation of activity. Again one group of lesions would develop by more or less regular periods of growth interrupted by short intervals of inaction, while another would show the greatest degree of irregularity. As a rule, a period of growth lasted for a week or more before it was interrupted by an interval of inactivity.

The extent of the change which took place during one of these periods of growth or regression was also a matter of great variation. Growth might be rapid or extremely slow, so rapid that a chancre measuring 2 or more cm. in diameter would develop within as many weeks from the commencement of the reaction, or so slow that no change could be detected from week to week and growth was recognizable only by the change which took place from month to month. Conversely, the phase of regression might be limited to inaction only or might go so far as almost to obliterate the lesion which had been developed and still be followed by an active renewal of growth. However, marked changes in one direction followed by marked changes in the other were extremely uncommon except in cases in which the complete life cycle of the lesion consisted of one such series of changes or was the terminal change in the local infection.

While the extent of the cyclic change was usually limited to a moderate reaction in one direction or the other, the number of such cycles was at times very great, especially in lesions which developed slowly and persisted over long periods of time. As a rule, however, the complete series of such cycles did not exceed three or four and not infrequently was limited to a single cycle.

The duration of the infection in the scrotum as a local or primary focus of infection may be variously stated as from 1 to 18 months. In a limited number of cases the infection developed quickly and subsided with equal rapidity or there was but slight local reaction and this soon subsided. At the other extreme, there were infections which remained firmly established for more than a year, but the average duration of the scrotal chancre was hardly more than 4 to 6 months.

In the majority of instances, the infection terminated by degrees. Development gradually ceased and after remaining in a more or less stationary condition for a time, the lesion gradually underwent resolution or healing with the production of a scar, the process consuming on an average from 4 to 6 weeks.

Spirochete Content of Scrotal Chancres.

To demonstrate the presence of spirochetes by the aspiration of fluid from scrotal lesions is usually a very simple operation, but owing to differences in the character and structure of the lesions, comparisons of the numbers of spirochetes in different examples or in different portions of the same lesion are always open to some question. Thus spirochetes are obtained with comparative ease from lesions of a cellular or mucoid character but are more difficult to obtain from fibrous lesions. Again, calculations may be considerably upset by the simple element of dilution which becomes of especial importance where edema exists, and in any case, a negative examination has only a relative significance.

It seems well to emphasize the importance of these points, since in therapeutic experiments, so much stress has been laid upon the number of spirochetes present in lesions at the time of treatment and upon the relative reduction in the number of spirochetes produced by different therapeutic agents. From a wide experience, we realize that no small part of such differences may be traceable to just such factors as those which have been enumerated. This is especially applicable to effects attributed to drugs which produce a marked increase in the fluid content of lesions as a characteristic feature of their action, and, we may add, there are many such drugs, and these are the ones which, as a rule, produce the most striking apparent reductions in the number of spirochetes present.

With these facts in mind, it may be said that during the early period of chancre growth and as long as a uniform and continuous growth was maintained, actively motile spirochetes could be found in abundance throughout the lesion. Eventually the spirochete content became variable as in other primary lesions. They were numerous and actively motile during periods of active growth or even quiescence, but decreased or even disappeared temporarily from the aspirated fluid during periods of actual regression.

Spirochetes varied likewise with developmental processes or with pathological alterations taking place in different portions of the lesion. As central necrosis developed, they became less numerous towards the center of the lesion or less active and tended to accumu-

late in greatest numbers towards the outer or growing edge. In well developed lesions with necrotic but firm fibrous centers, spirochetes were obtained with difficulty from the central zone and might be obtained only from the outer shell of living tissue. However, in chancres which underwent central softening following necrosis, spirochetes were at times quite numerous in the necrotic debris.

With the progress of the infection and the appearance of irregularly distributed areas of growth and of necrosis, the distribution of spirochetes became likewise irregular; they tended to be numerous and actively motile in regions of most active growth, less numerous and less active in portions of the lesion which were inactive or regressing.

The relation of the spirochete to the size and character of the lesion was less definite. Spirochetes were as abundant and as actively motile in some of the smallest and most insignificant lesions as in the largest and most typical lesion. As a rule, spirochetes were obtained in greater numbers by aspiration of cellular and mucoid lesions or portions of lesions than from fibrous lesions or areas, but, as previously stated, this does not necessarily represent actual differences in the spirochetal content of the respective lesions.

Results Obtained from Scrotal Inoculation.

Before concluding the subject of the scrotal infection in the rabbit, it seems well to refer briefly to the results which one may obtain from scrotal inoculations with *Treponema pallidum* and certain factors which influence these results.

As has already been indicated, there is no particular difficulty in obtaining an infection with organisms which have been thoroughly adapted to the rabbit. With such organisms as we have used, 100 per cent of takes can be obtained with perfect regularity provided one observes a few simple precautions which concern chiefly the state of the virus, the animals used, and the technique of inoculation. As regards the character of the local infection, or more properly the local reaction, uniformity is more difficult to attain, but this also is influenced to a considerable extent by the same factors.

It has been found that the first essential to success is the use of a suitable virus. As far as mere infection is concerned, material taken from any lesion containing a fair number of actively motile spirochetes will produce infection, but the character of the infection will vary according to the state of the lesion or of the spirochetes in the lesion. Other conditions being equal, the local reaction will take place more promptly and large actively growing chancres will be produced with greater regularity when the material for inoculation is taken from a fresh actively growing lesion, preferably before the height of the first cycle of reaction has been reached. Material taken from lesions during the ascending phase of the second cycle of an orchitis or of later cycles in the case of skin lesions will sometimes give good results, but at other times, the results are apt to be irregular and less satisfactory as far as the production of chancres is concerned.[1]

In addition, it should be noted that certain strains of *Treponema pallidum* will give results which are more constant and more satisfactory than the results obtained with other organisms.

The second factor in the production of scrotal chancres is the character of the animal used. The proverbial large rabbit or rabbit with large testicles which is usually interpreted as an old rabbit with a more or less ample or redundant scrotum is a poor subject for the production of scrotal chancres. The best animals are those just approaching full maturity with well developed but not necessarily large testicles and a thin delicate scrotum. It is also desirable that the rabbits used be well nourished and free from disease.

Among the various breeds of rabbits, there are great differences in the scrotal reaction to *pallidum* infections. The Belgian and Flemish giants in particular give poor results, while the small albinos, grays, browns, and Dutch belts on the whole give results of the most satisfactory character.

[1] It is not to be inferred that the extent of the local reaction in the scrotum is an index of the severity of the infection as a whole, for, as has been pointed out, some of the most severe generalized infections follow the most insignificant local reactions. Further, there is evidence to the effect that in general a pronounced local reaction in the testicles or scrotum of the rabbit inhibits the development of other focal infections. This subject will be dealt with in detail in a subsequent paper.

Technique is another factor which plays some part in the success of scrotal inoculations, and the chief element here is simplicity. No antisepsis is necessary, and the use of strong antiseptics is distinctly contraindicated both on account of irritant action upon the scrotum and the possible effect of these substances upon the spirochetes themselves. Many failures in scrotal inoculations have been directly attributable to unnecessary precautions in this direction. Cleanliness, careful operation, and avoidance of undue trauma are the three essentials.

With the use of a suitable virus, good animals, and proper technique, one may attain a high degree of uniformity from scrotal inoculations in as far as the percentage of takes and the production of typical scrotal chancres is concerned. Large indurated chancres were frequently obtained by us in from 75 to 100 per cent of the animals inoculated, but as a rule such lesions were produced in not more than 50 to 75 per cent of the animals. The factor of individuality in the reaction to infection cannot be entirely overcome in *pallidum* inoculations. Just as the typical chancres in a given series of animals will differ from one another, so also one may expect to obtain all degrees of variations in the response of individual animals to a constant set of conditions of inoculation, and irregularities will appear in the results in spite of all that can be done to prevent them.

CONCLUSIONS.

A study of the local infections produced by *Treponema pallidum* in the testicles and in the scrotum of the rabbit leads to the conclusion that the phenomena of infection in the two cases are essentially the same and that such differences as do exist are attributable to differences in the character of the two organs. Upon the basis of these studies, it is possible, therefore, to extend the conclusions which have already been reached in regard to the nature of the local or primary reaction to infection with *Treponema pallidum*.

One is accustomed to think of this reaction as essentially a process of infiltration and proliferation and of the chancre as a circumscribed, indurated, granulomatous lesion. From a consideration of the facts derived from a study of the local infection in both the testicles and the

scrotum, it is apparent, however, that in the rabbit at least, this conception of the syphilitic infection is true only in part and that it is derived more from a study of a condition accomplished than from the process concerned in the production of this condition.

It would seem that there are concerned in the local reaction to infection an element of toxic injury with subsequent degeneration and necrosis which affects especially the lymph and blood vascular systems, a process of exudation and infiltration, and finally proliferation and a mass necrosis due to a progressive destruction of the vessels supplying the affected area. While infiltration, proliferation, and necrosis are the most noticeable features of the local reaction in the usual case of infection, they are, however, no more characteristic than the other processes mentioned and appear to be phenomena of secondary character and importance.

None of these changes bears a fixed relation to the infection, but they are subject to the widest possible variations and in consequence give rise to lesions of the most diverse character in all of which evidences of the same fundamental processes are to be seen. As one feature or another of the reaction becomes more marked, the character of the lesion changes accordingly. Thus the lesion produced in different animals or in the same animal at different periods of the infection may range from lesions in which congestion, edema, and even hemorrhage are the most prominent characteristics to massive granulomatous lesions on the one hand, and from diffuse or ill defined patches of infiltration with desquamation or exfoliation of the surface epithelium to the most sharply circumscribed and intensely indurated nodules on the other.

Finally, it may be said that there is nothing so far as we have been able to discover which clearly differentiates the local reaction at the primary focus of infection from localized reactions to a generalized infection, unless it is the one element of the intensity of the reaction. In many instances, even this distinction is lost, and, as will be brought out in subsequent papers, one reaction is but a repetition of the other, modified to a greater or less extent by the general reaction opposed to the infection and the character of the tissues within which the reaction takes place.

SUMMARY.

From a study of the reaction to scrotal inoculation with *Treponema pallidum* in a large series of rabbits, it was found that the specific reaction presented the following characteristics.

In general, the reaction in the scrotum became apparent within 7 to 14 days after inoculation but was subject to considerable variation. The early reaction took the form of an edematous swelling and congestion associated with a new growth of vessels or of an infiltration with more or less proliferation of fixed tissue cells. These reactions were either confined to a small circumscribed area of the scrotum or were of a diffusely spreading character, and as the infection advanced, the infiltration and proliferation together with such secondary changes as exfoliation, necrosis, and ulceration became the most conspicuous features of the reaction.

The course of the reaction in the scrotum was essentially the same as that in the testicle; that is, it was periodic in character and was marked by a phase of active progression followed by quiescence or regression and renewed activity.

The scrotal reaction resembled that in the testicle also in the varying character of the reaction, appearing at times as a circumscribed focus of reaction and later becoming diffuse, or first as a diffuse reaction which subsequently became more localized.

The lesions produced in consequence of this reaction were of two general types—one a circumscribed indurated granulomatous lesion closely resembling the human chancre, the other a diffuse infiltration more analogous to the secondary skin lesions of man. Both groups of lesions presented the greatest degree of individual variations and possessed no fixed status but were subject to frequent and marked transformations. After a period of from a few weeks to many months, the lesions in the scrotum disappeared spontaneously.

EXPLANATION OF PLATES.

All the illustrations are reproductions of untouched photographs which represent the objects at their natural size. The statements of time given are estimated from the date of inoculation except where otherwise stated.

PLATE 83.

FIGS. 1 to 6. Typical nodular chancres of the scrotum.

FIG. 1. 49 days. Characteristic multinodular chancres with small depressed ulcers.

FIG. 2. 41 days. Intensely indurated chancres with marked central necrosis. Edema and congestion of the scrotum with focal hemorrhages on the left.

FIG. 3. 44 days. Nodular chancres with unusually marked central necrosis and only a comparatively thin shell of living tissue. These features were developmental characteristics.

FIG. 4. 44 days. Large nodular chancres with unilateral flattening. Growing surfaces slightly mucoid in character. Edema of the scrotum.

FIG. 5. 44 days. Large nodular chancres with marked surface flattening of the right chancre due to spreading necrosis.

FIG. 6. 70 days. A vigorous nodular chancre on the left with marked central necrosis of the chancre on the right—a phenomenon of decadence.

PLATE 84.

FIGS. 7 to 12. Nodular chancres of a more spherical type with a lessened tendency to necrosis and ulceration.

FIG. 7. 43 days. Spherical chancres. Spreading surface necrosis on the right, slight necrosis on the left.

FIG. 8. 46 days. Spherical chancres ulcerated and unulcerated.

FIG. 9. 60 days. Spherical chancres. The skin surface on the left still uninvolved.

FIG. 10. 90 days. Irregular nodular chancres with small areas of skin necrosis.

FIG. 11. 89 days. Spherical chancres in the subcutaneous tissues of the scrotum, with no sign of surface necrosis. Diffuse infiltration of the lower end of the scrotum on the left.

FIG. 12. 112 days. Spherical chancres in the deeper layers of the skin with infiltration of the papillary layers but no necrosis.

PLATE 85.

FIGS. 13 to 18. Typical lenticular and discoid chancres of the scrotum.

FIG. 13. 28 days. Rapidly growing lenticular chancres with spreading necrosis of the skin surface. Thick acuminate crusts.

FIG. 14. 28 days. Discoid chancres with deep necrosis and ulceration on the right.

FIG. 15. 38 days. Discoid chancres with deep ulceration and thick, dry crusts. Marked edema of the scrotum.

FIG. 16. 45 days. Typical mucoid chancre with extensive ulceration and infiltration of the skin.

FIG. 17. 39 days. Discoid chancre of more fibrous type.

FIG. 18. 46 days. Discoid chancres with spreading necrosis and thick imbricated crusts.

PLATE 86.

FIGS. 19 to 21. Growth and necrosis of mucoid chancre.

FIG. 19. 45 days.

FIG. 20. 65 days.

FIG. 21. 86 days.

FIGS. 22 to 24. Evolution of a more spherical mucoid chancre.

FIG. 22. 39 days.

FIG. 23. 46 days. Necrosis beginning to spread.

FIG. 24. 60 days. Spreading necrosis with thick imbricated crusts.

PLATE 87.

FIGS. 25 to 30. Modified types of lenticular and discoid chancres.

FIG. 25. 35 days. Lenticular mucoid chancres.

FIG. 26. 40 days. Mucoid chancres tending towards a spherical form.

FIG. 27. 61 days. Lenticular chancres of dense fibrous structure.

FIG. 28. 65 days. Lenticular chancres of dense fibrous structure showing marked necrosis and thick adherent crusts.

FIG. 29. 37 days. Mucoid chancres with moderate induration, slight skin involvement, and undermining necrosis (right).

FIG. 30. 97 days. Old fibrous chancres with undermining necrosis and relaxed skin covering. Inactive.

PLATE 88.

FIGS. 31 to 36. Transitional types of chancre reactions.

FIG. 31. 84 days. Right, a flattened plaque of induration with ulceration extending practically to its edges. Left, an irregular area of induration, necrosis, and ulceration. Both chancres still quite active.

FIG. 32. 73 days. An irregular ulcerated nodule on the right and a small flattened chancre on the left.

FIG. 33. 55 days. Irregular mass of induration on the right and a very small but typical lenticular chancre on the left.

FIG. 34. 36 days. Small lenticular chancre on the right and a small patch of necrosis in the skin on the left.

FIG. 35. 58 days. Irregular patches of necrosis and ulceration with slight infiltration of the surrounding tissues.

FIG. 36. 29 days. Small nodules of induration.

PLATE 89.

FIGS. 37 to 42. Atypical scrotal lesions (chancres (?)).

FIG. 37. 43 days. Right, a small indurated papule with central ulcer. Left, an area of diffuse infiltration and slight desquamation of epithelium.

FIG. 38. 77 days. Diffuse infiltration with superficial necrosis and exfoliation.

FIG. 39. 136 days. Marked infiltration of certain areas of the scrotum with focalized areas of necrosis and ulceration and extensive exfoliation.

FIG. 40. 190 days. Diffuse infiltration of the scrotum with marked exfoliation in certain areas.

FIG. 41. 94 days. Diffuse infiltration of the scrotum, secondary ulceration with slight induration, and double orchitis.

FIG. 42. 316 days. A late reversion of a diffuse scrotal syphilis to lesions of a circumscribed type.

(Brown and Pearce: Experimental syphilis in the rabbit. II.)

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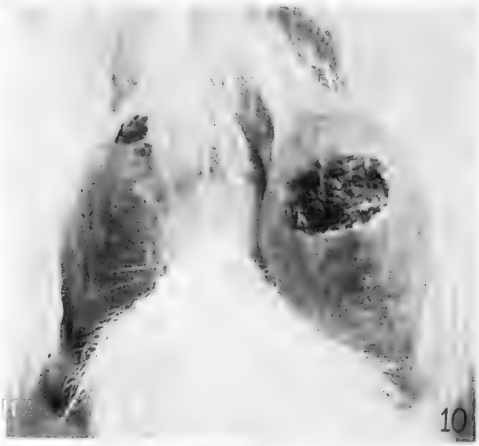

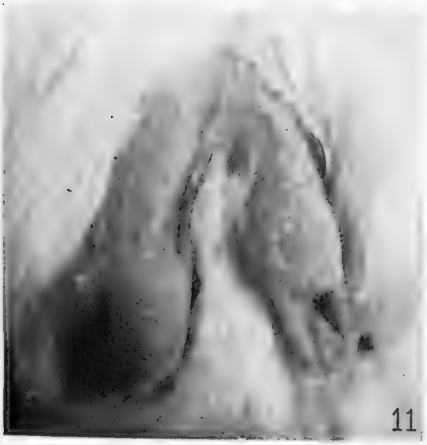

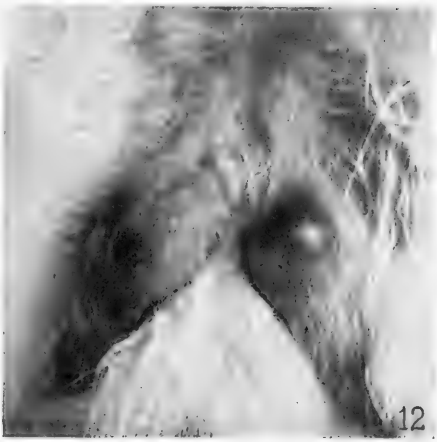

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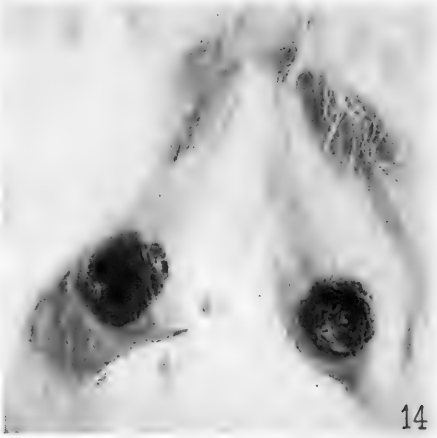
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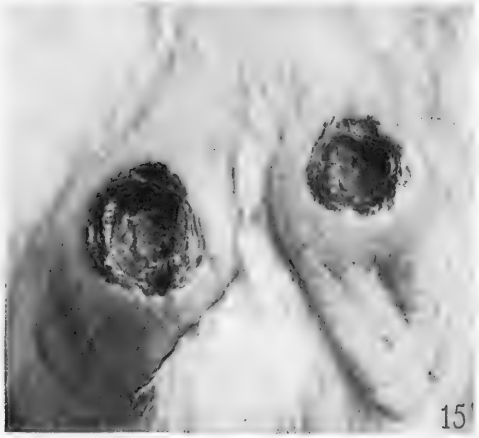
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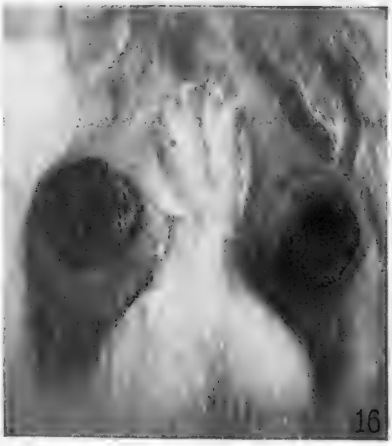
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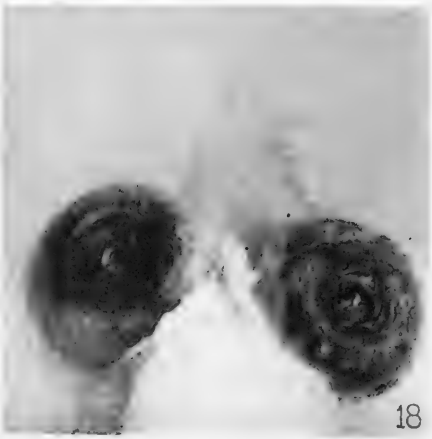
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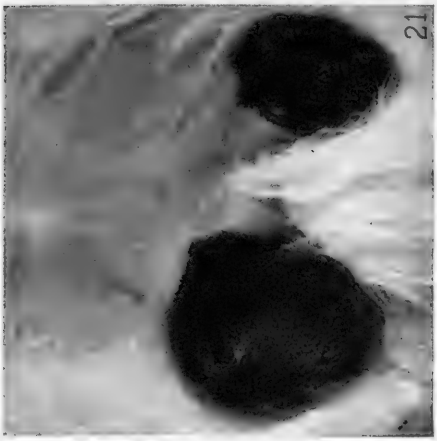

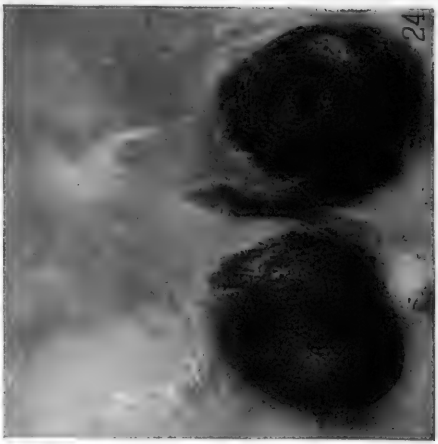

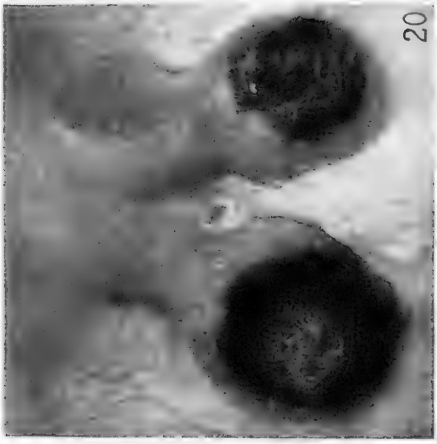

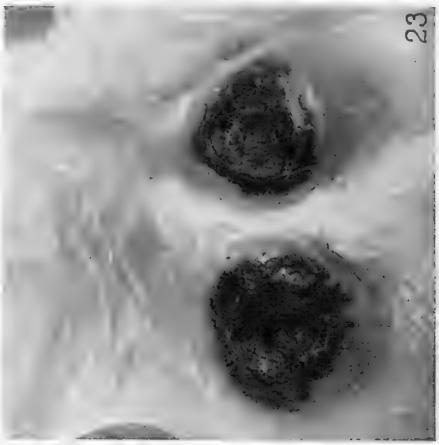

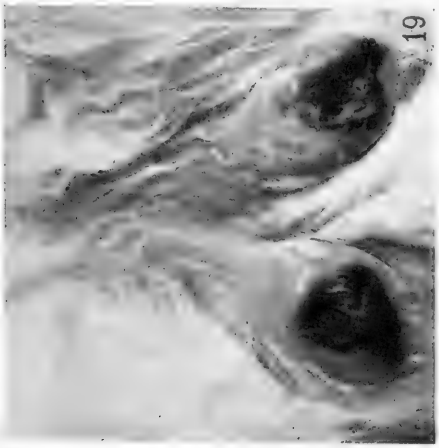

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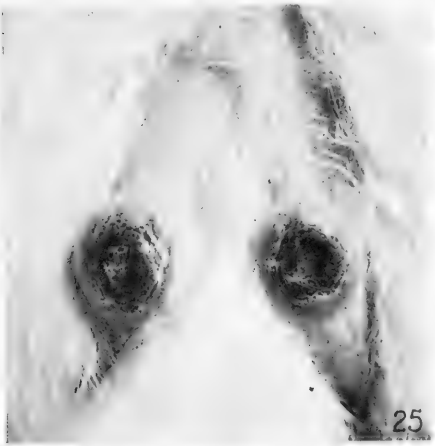
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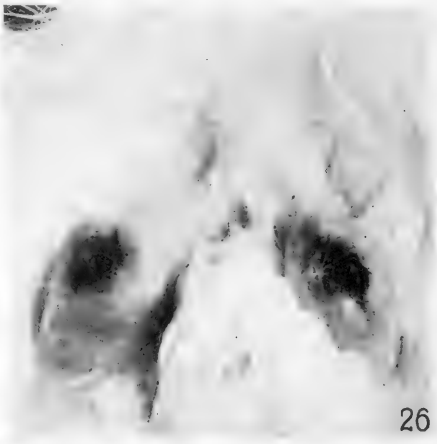
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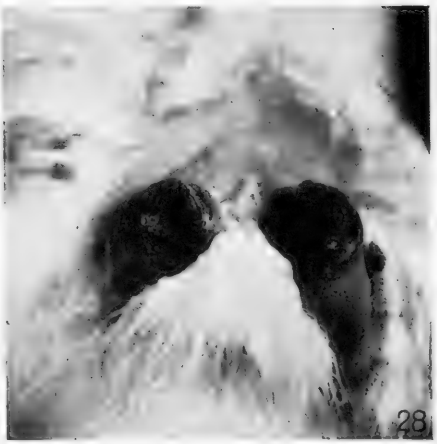
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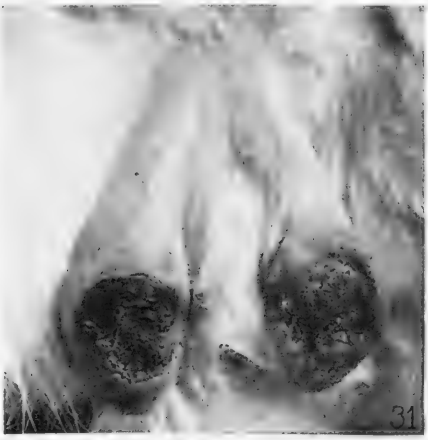

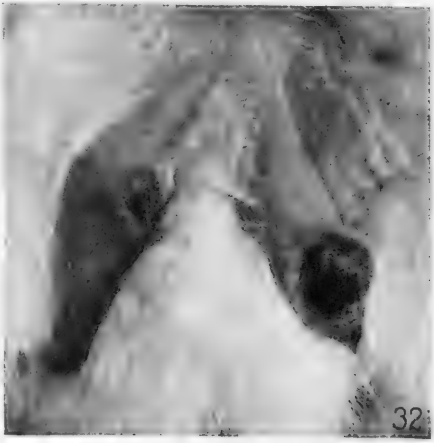

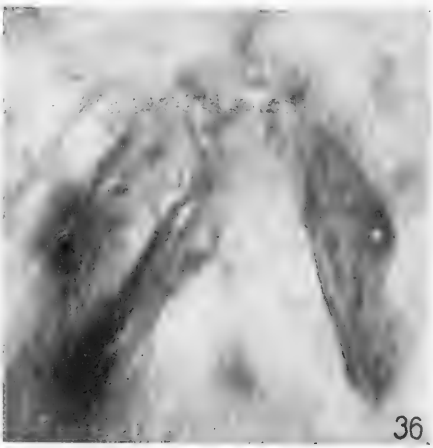

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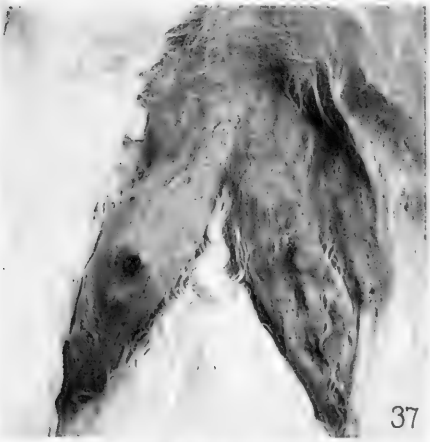

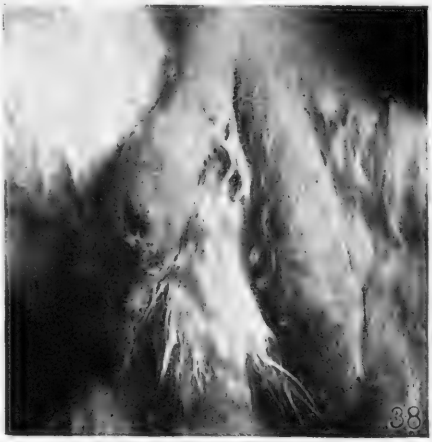

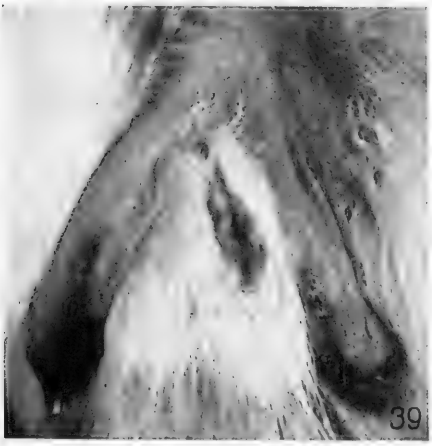

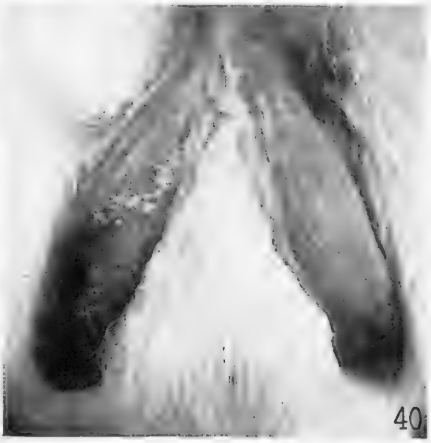

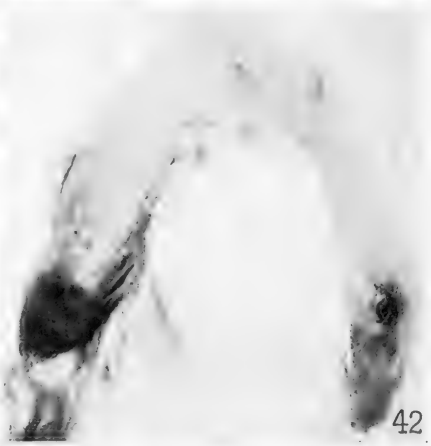

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EXPERIMENTAL SYPHILIS IN THE RABBIT.

III. Local Dissemination, Local Recurrence, and Involvement of Regional Lymphatics.

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(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plates 90 to 97.

(Received for publication, February 2, 1920.)

In preceding papers of this series, the testicular and scrotal infections of *Treponema pallidum* were described from the view-point of a progressive local infection, and in following out this mode of presentation, a number of lesions were described as transformations or extensions of the local infection without any attempt to differentiate such lesions from lesions of a similar character which might arise as a result of local dissemination of the infection rather than from direct extension or transformation of primary lesions. It was pointed out, however, that there were many lesions of the testicles and scrotum which occupied a position of uncertainty with reference to the primary infection and that it could not be determined in all cases whether the lesions present represented actual transformations and extensions of a primary infection or had arisen as a result of local dissemination.

In brief, it was recognized that there were two groups of lesions in the testicles and scrotum which could not be clearly separated from one another. One group is composed of modified and persistent forms of primary lesions together with direct extensions and true recurrences, while the other represents lesions which owe their origin to local or regional dissemination of the virus.

These lesions are of especial interest in that they mark a transition between conditions which can be clearly recognized as primary manifestations of infection and conditions which represent true generalized infection. The subjects of chief interest in this connection are those of local spread and local dissemination of the infection, recurrent and

consecutive lesions, and involvement of regional lymphatics. References to this phase of experimental syphilis have been very few and for the most part rather vague.

Attention was called to the phenomenon of local metastasis by Uhlenhuth and Mulzer and by Truffi at about the same time. Truffi (1) reported a case of a granulomatous nodule arising in the epididymis and another of a lesion in the tunics following primary infections of the scrotum. Uhlenhuth and Mulzer (2) reported metastatic infection from one testicle to the other testicle and scrotum and a second case of metastasis to an uninoculated testicle after removal of the inoculated testicle. These observations were made in 1909 and 1910, and since that time, metastases of this kind have frequently been cited as evidence of generalization of the infection in the experimental animal.

Involvement of the inguinal lymph nodes in infected animals has likewise been noted both as an analogy to the human infection and as proof of generalization. This condition was first reported by Ossola (3) (1909) who was able to demonstrate spirochetes in sections of the inguinal nodes of animals inoculated in the scrotum. These findings were confirmed by Truffi (4) and the virulence of the organisms was proven by animal inoculation.

Other than observations of this kind, very little has been recorded concerning this border-line group of syphilitic manifestations.

Local or Regional Dissemination.

In animals infected with *Treponema pallidum*, one must recognize the existence of two opposing influences, the tendency of the organism to spread beyond the local confines of the point of inoculation and the opposing reaction on the part of the animal. Some extension of the infection and of the zone of local reaction occurs in all animals even with the most sharply circumscribed lesions, but the extent of the two processes does not run parallel.

The details of the local changes are either masked in a continuous and widespread reaction or else there are no lesions to mark the extension of the infection beyond the zone of primary reaction. In exceptional instances, however, cases do arise which when linked together furnish some interesting data concerning the spread, or dissemination, of the infection within the regions adjacent to the point of inoculation.

In addition to the widespread reaction commonly observed in the course of development of primary lesions, there were three general

types of local reaction which we were able to make out in connection with the dissemination of the infection in the testicles and scrotum: first, a diffuse reaction with the formation of multiple foci of secondary reaction arising subsequent to the development of the primary lesion; second, a reaction characterized by the formation of lesions having a perivascular arrangement; and third, dissemination of the infection with the production of multiple lesions showing no demonstrable connection with the primary focus of infection. While lesions of these three classes occurred in both the scrotum and testicles, they were most clearly definable in the scrotum, and for that reason, the discussion of the subject will be confined to the scrotal infection.

Diffuse Secondary Reaction with the Formation of Multiple Focal Lesions.—One of the most common forms of local dissemination observed was one which took place with a more or less diffuse reaction about a primary focus of infection and led to the formation of multiple secondary lesions which tended to become fused into a single chancre-like mass. This form of reaction is apt to be confusing and it is only in cases where the successive steps in the process can be followed that the ultimate lesion can be distinguished in any way from a true primary lesion. A typical case of this kind is illustrated in Figs. 1 to 3.

The primary lesions in this animal were small circumscribed chancres of a rather indolent character and for approximately 2 months there was nothing unusual about the local infection. A slight diffuse thickening of the scrotum then developed about these lesions and a number of small thickened plaques were formed here and there in the scrotum. These plaques developed into nodules and the diffuse reaction in the scrotum increased after the manner shown in Fig. 1. At the time this photograph was taken, 85 days after inoculation, the primary lesions themselves (indicated by arrows) had begun to grow actively; the entire scrotum was markedly thickened and slightly reddened, and the focal lesions showed signs of necrosis and ulceration. The development of these lesions continued as indicated by Figs. 2 (91 days after inoculation) and 3 (113 days after inoculation) with the formation of an increasing number of secondary foci which tended to fuse into a single large granulomatous lesion indistinguishable in character from an ordinary chancre.

It should be noted here that the development of this series of lesions in the scrotum, occurring 2 months after inoculation, coincided with the appearance of similar granulomatous lesions on the

feet and legs of this animal and the two sets of lesions ran a parallel course of development.

This is but one of many cases of the kind which have come under our observation but will serve to indicate the possibilities for confusion of primary lesions and lesions which owe their origin to dissemination from a primary focus of infection.

Local Dissemination with the Formation of Perivascular Lesions.—The second form of local dissemination to be considered differed from the first chiefly in that the development of the lesions was less obscured and the lesions themselves were of a very distinctive type.

In taking up this group of lesions, we may refer first to a form of direct extension from the primary lesions which was observed in cases in which the initial lesion was slow in developing or of slight extent and spread of the infection along the perivascular tissues could be detected even before the reaction at the site of inoculation had become well established. The animal shown in Fig. 4 was one of a group inoculated with a virus known to contain large numbers of spirochetes of low vitality. Following inoculation, the implant became soft and cheesy in character and no specific reaction could be detected for about 6 weeks. Finally, a narrow line of induration developed at the outer margin of the implant in the right scrotum and fine thread-like lines of infiltration immediately began to spread along the course of the blood vessels leading away from this area as shown in the photograph (Fig. 4) taken 56 days after inoculation. These lines of infiltration grew until they formed a series of cords about 1 mm. in diameter with node-like thickenings at various points. Later, the intervening sections in the distal portions of the cords disappeared, leaving a series of isolated shotty nodules, while the parts nearest the implant became fused into a single irregular granulomatous mass.

In the left scrotum, a similar type of reaction took place except that the lines of perivascular extension were not so clearly defined. When first discovered, there were numerous isolated points of induration diffusely scattered through the scrotum, many of them occupying a perivascular position. Soon after the appearance of these disseminated lesions in the scrotum, lesions developed upon the sheath and about the anus of this animal.

We have in this animal what appears to be a twofold process, first a gradual but direct extension of the primary reaction along certain definite lines, and second a widespread development of focal lesions resulting from dissemination of spirochetes with which the development of the primary lesion had not kept pace.

A somewhat clearer example of local dissemination with the production of perivascular lesions is to be found in the animal shown in Figs. 5 and 6 which belonged to the same series as that shown in Figs. 1 to 3. The initial lesions were again small ulcerated chancres with slight induration. These lesions were soon transformed into a moderate diffuse thickening of the ventral surface of the scrotum, while the ulcers healed, leaving small puckered scars (Fig. 5). About 6 weeks after inoculation, a series of small shotty nodules was palpable in the dorsal fold of the scrotum. These nodules were just visible as translucent points distributed in chains along the course of the blood vessels extending centrally from the thickened patches in the scrotum. They gradually increased, and at the time the first photograph was taken (Fig. 5, 63 days after inoculation), there were numerous nodules distributed all through the scrotum, including the area beneath the scar, and the course of the blood vessels in the dorsal fold was marked by a series of indurated lines and nodes (Fig. 5). While these lesions are shown on only one side, the condition on the other was exactly the same.

During the next 3 weeks, the character of the lesions changed materially. Many of the papular lesions disappeared, leaving only a few perivascular lesions in the dorsal portion of the scrotum (Fig. 6). The chief seat of the specific reaction shifted back to the ventral surface of the scrotum which showed a heavy shell of doughy thickening with a few large masses of induration. The subsequent changes were much the same as those shown in the first series of photographs (Figs. 1 to 3).

Again we may note that the occurrence of these papular lesions in the scrotum coincided with the development of a periosteal lesion at the distal end of the left ulna.

While lesions of the type described were observed in all parts of the scrotum, the seat of predilection was the lower end of the dorsal fold, and the occurrence of one or more lesions in this location was quite common. When the lesions were multiple or in the form of cords, they frequently followed a definite line of distribution or extension from the point of inoculation or from the primary lesion towards the body axis.

Local Dissemination with the Formation of Multiple Secondary Lesions.—The third group of disseminated lesions to be described differed from the first group only in that they were clearly focalized lesions from the beginning and from those of the second group in that they showed no apparent perivascular relation and no definite connection with the parent lesion. These lesions usually appeared as multiple papules or plaques and tended to develop into multiple lesions of a chancre-like character. Sometimes there were only one

or two such lesions, but at others, there were as many as six or eight. The animal shown in Fig. 7 had six distinct lesions of different sizes and stages of development in each scrotum, among which it would be rather difficult to identify the primary lesion. As in the previous cases, they tended ultimately to diminish in number, and the reaction usually became centered in one or two lesions which developed to a considerable size, overgrowing and fusing with the surrounding lesions.

It is probable that lesions of this class have in general been regarded as multiple primary lesions, but as a rule they do not begin to appear until after a primary lesion of some sort has developed at the immediate point of inoculation or for some 6 weeks or more after inoculation, and while they may become the main lesions present, they are not primary in the sense of lesions developing as a result of simultaneous localization of the infection. This distinction may or may not be of importance according to the significance which may be attached to primary and so called secondary lesions.

Recurrent Lesions.

The lesions which develop in the testicles and scrotum of an infected animal subsequent to the regression or healing of an initial lesion have many points of interest, but their chief importance lies in their bearing upon problems of local dissemination and the persistence of active infection when once the local lesions have completely disappeared. In its application to experimental therapy, there are few subjects of greater importance than this, and while we cannot enter into this phase of the subject with the thoroughness which it demands, the therapeutic importance of recurrent lesions may be kept in mind.

The lesions which develop after spontaneous healing of an initial lesion and those which appear after healing which has been induced by artificial means are identical in all respects. Induced healing, as by the use of therapeutic agents, offers the advantage, however, of clearer definition between the process of healing and recurrence, and for this reason we shall use cases for illustration in which the healing of the original lesion was experimentally induced.

As an introduction to the subject of true recurrence, it may be pointed out that the power of recovery in an early and actively developing scrotal chancre is very great and that regression may be carried almost to the point of complete healing and be followed by prompt regeneration of the lesion. This fact is illustrated in Figs. 8 to 10. Fig. 8 shows two chancres 40 days after inoculation at which time the animal was treated. The lesions regressed rapidly, and 2 weeks later, there was an irregular thickened mass in the right scrotum and only a small ulcer with no induration in the left (Fig. 9). At this point, regeneration set in, and in 2 weeks more, chancres showing the same characteristics as the original lesions were produced (Fig. 10). This is the result usually obtained when regression stopped short of complete healing.

Once complete healing of a lesion was accomplished, it was rare that another lesion developed from exactly the same point. The four most common locations for recurrent lesions were the tissues beneath the scar, the edges of the scar, the dorsal folds of the scrotum, and the testicles. The lesions developing in the scar were usually no more than indurated plaques, although typical chancres were observed which formed upon the basis of an old lesion.

In the tissues beneath the scar, the recurrent lesions usually took the form of indurated nodules, some of which might develop to considerable size and involve the overlying skin with the formation of chancre-like lesions.

A typical example of this form of recurrence is given in Figs. 11 to 13. Fig. 11 is the original chancre 29 days after inoculation, Fig. 12 the healed lesions, and Fig. 13 the multinodular recurrence with an ulcerated chancre-like lesion in the left scrotum 78 days after treatment.

A more common form of deep scrotal recurrence is that shown in Figs. 14 to 16. Recurrence in this animal did not take place until 55 days after treatment, and the lesions formed were small indurated nodules which were at first confined to the subcutaneous tissues. Later, the skin became involved, and a diffuse induration with multiple focal lesions extended over a large portion of the scrotum of both testicles (Fig. 16). During 4 months observation, no typical chancre-like lesion developed.

Another form of recurrence which was frequently seen in the scrotum is that shown in Figs. 17 to 19. This animal was treated 37 days after inoculation and the chancres healed promptly. 55 days after treatment, there were a few small indurated nodules at the outer edges of the scars on both sides. The scars themselves were smooth and thin (Fig. 19, 77 days after treatment). Subsequently other lesions of the same character appeared elsewhere in the scrotum, but they remained for only a short time and none of them developed to a size much larger than that shown in the photograph.

In some instances these recurrent lesions even though they were far removed from the seat of the original chancre showed a form of development identical with that of a chancre. Such a case is illustrated in Figs. 20 to 22. Recurrence took place in this animal 127 days after treatment, and the lesions appeared as single, minute, indurated nodules situated at the lower end of the dorsal fold of the scrotum; they were bilateral and symmetrical. These lesions grew rapidly and assumed the appearance of typical chancres (Fig. 22, 133 days after treatment).

In addition to the various forms of nodular lesions described, diffuse lesions of various types were observed in many animals. A case of this type which combines something of the characteristics of a diffuse and a focal process is shown in Figs. 23 to 25. This animal was treated 48 days after inoculation (Fig. 23). The lesions regressed very rapidly until they had almost healed, when a diffuse infiltration appeared over a large part of the scrotum (Fig. 24). Healing of the initial lesion continued, however, with the formation of an ordinary looking scar, and the thickening of the scrotum diminished (compare Figs. 24 and 25). 28 days after treatment, a series of slightly reddened and translucent points appeared around the margins of the scars (Fig. 25, 35 days after treatment). These spread rapidly, producing a slight diffuse induration in the scrotum with scaly points here and there.

A final form of recurrence to be noted is that which takes place in the testicle following the healing of a scrotal lesion, and it may be mentioned in this connection that such recurrences were quite common as were recurrences of testicular lesions in the scrotum. The most common locations of recurrent lesions in the testicle were the head of the epididymis, the tail of the epididymis, the mediastinum testis, and the tunics, or the same positions in which primary testicular infections tend to become localized.

This form of recurrence is illustrated in Figs. 26 to 28. The chancres in the scrotum of this animal healed completely in 3 weeks. 1 week later, there was a definite swelling of both testicles and indurated nodules were found in the head of the epididymis on both sides (Fig. 28); the scrotum remained normal.

The time of occurrence and the relative frequence of the various types of recurrent lesions will not be entered into. It seems well to mention, however, that the more characteristic lesions usually appeared early after the healing of the original lesion, while the smaller, less easily recognizable ones were more delayed in their development. The time of recurrence in our series of animals varied anywhere from a few days to many months, and so many circumstances enter into

estimations of the possibilities of local or regional recurrence that definite statements as to time limits cannot be made upon the basis of the data at present available.

To emphasize the difficulties attendant upon observations as to recurrence of local infections, the case illustrated in Figs. 29 to 31 may be cited. Following treatment, the chancres in this animal (Fig. 29) healed rather slowly, and healing was not complete at the end of 6 weeks (Fig. 30); however, smooth, thin scars were eventually formed. 63 days after treatment, two small areas of thickening appeared at the edge of the scar in the right scrotum but disappeared within a few days and infection could not be proven. 105 days after treatment, the extremely small nodule shown in Fig. 31 appeared in the left scrotum and proved to be syphilitic. There was a slight local edema associated with this nodule, but the nodule did not increase in size and persisted for only a short time. No other local lesions were found, but the animal developed a specific iritis 192 days after treatment.

A second animal of a similar character which came under our observation was one in which minute points of induration appeared in the dorsal folds of the scrotum 107 days after treatment. These nodules persisted for 8 months with very little change and no other lesions appeared for some time, but eventually a generalized rash developed upon the face and ears.

It will be noted that the lesions described as recurrences are not unlike some of the conditions previously described in connection with transformations of primary lesion and especially like those resulting from local dissemination. In fact, transformation, dissemination, and recurrence are all overlapping processes with no sharp line of separation between them, and each of these phenomena throws some light upon the others.

Involvement of Regional Lymphatics.

Enlargement and induration of the inguinal lymph nodes has been noted by several observers in connection with *pallidum* infections of both the testicles and the scrotum of the rabbit, and organisms have been demonstrated in these nodes in some instances. There is, however, little definite information concerning the relation of lymphadenitis to the experimental infection. The subject is a very broad one in itself and will not be discussed in detail at the present time. This aspect of the problem of experimental syphilis will be taken up in a separate paper, and we shall confine ourselves here to

general statements concerning involvement of the inguinal nodes in primary infections of the testicle and scrotum as a phase of local dissemination.

In normal rabbits, the inguinal glands are small, ovoid, or flattened masses which are barely visible or palpable (Fig. 32). From examination of a large number of rabbits, it was found that enlargement and induration of these nodes was inconstant in testicular infections and depended largely upon the extent of the local infection. In cases in which localized infection was confined to the testicles proper, there was little or no involvement of the inguinal glands, but when the infection extended to the scrotum, these glands became involved just as in cases of primary scrotal infection.

Character of the Involvement.—Localized infection in the scrotum was found to be invariably associated with marked inguinal lymphadenitis. Following inoculation or extension of an infection to the scrotum, pathological changes in the inguinal nodes could be detected almost immediately. The change began as an acute swelling, part of which might have been due to a non-syphilitic reaction but was merged indistinguishably with the syphilitic reaction which progressed rapidly until the affected glands were several times their normal size. During the early stages of the enlargement, the glands remained rather soft but gradually became harder and finally reached a condition of extreme induration. In some instances, the stage of acute swelling was absent; in these cases, the glands first became hard and shotty and then underwent a gradual enlargement.

The exact change which occurred in individual animals was found to be very variable, but a general idea of the inguinal adenopathy may be gained from an examination of the photographs reproduced in Figs. 32 to 43 which represent various forms and degrees of involvement.

As may be seen by reference to Figs. 33 and 34, a well developed adenitis was present in cases of active infection within a few days after inoculation. The 5 day example given in Fig. 33 is a rather pronounced instance of acute bilateral swelling more marked on the right than on the left. In Fig. 34 (7 days after inoculation), the change is not so apparent, especially on the right. Upon close examination, however, it will be seen that there are three nodes in this region, the largest of which is situated immediately over the cord and hence does not stand out so prominently as the one on the left.

The phase of acute swelling as exemplified in these two illustrations frequently reached its height by the end of the 1st or 2nd week after inoculation. The induration, however, continued to increase for some time and was not infrequently associated with a slight temporary decrease in the size of the gland. When fully developed, the inguinal adenopathy presented a variety of appearances. The glands chiefly affected were those immediately adjacent to the inguinal ring. As a rule, the involvement was well marked and of the same character on the two sides but rarely equal (see figures); occasionally the nodes of one side were much more enlarged and indurated than those of the other (Fig. 36). In some instances, the nodes affected were situated higher in the groin (Fig. 37), and even those of the flank might be enlarged and indurated.

Usually there were only one or two nodes involved on each side, and these were of a rounded or oval shape. Sometimes a number of small nodes were grouped together in one mass (Fig. 40); occasionally the nodes were flattened or lay in positions which made it difficult to appreciate the full extent of their enlargement. This may be seen by a comparison of the left inguinals in Figs. 41 and 42, in the latter of which the node has been picked up in order to show its actual size. This photograph also serves to convey some idea of the degree of induration in these nodes which when fully developed were extremely hard and of a semitranslucent appearance.

Significance of Lymphoid Involvement.—As will be noted by a comparison of the various figures, there was no apparent relation between the size of the scrotal lesion and the extent of the lymphadenitis. It frequently happened that the relative involvement of scrotum and nodes was in inverse proportion. The force of this statement is well brought out by Fig. 43 which shows a most pronounced lymphadenitis in an animal with comparatively slight scrotal lesions. Involvement of the inguinal glands appeared to be more an index of the activity of the scrotal infection and of the spread of the infection than of the extent of the local reaction in the scrotum.[1]

[1] In addition to the observations here recorded, this statement is based upon a long series of experiments in which the presence or absence of localized infection in the lymph nodes has been determined at periods of from a few hours after inoculation to more than 2½ years. The details of this work will be reported later. It is sufficient at present to say that enlargement and induration of lymph nodes in the rabbit always signify existent infection of the nodes.

As long as there was active infection in the scrotum, the inguinal nodes were apt to remain enlarged and indurated, but as the scrotal infection subsided, the nodes usually softened and diminished in size. Occasionally these changes occurred even before any very definite change in the scrotal lesions was noted, while in other instances, the adenopathy persisted even though the scrotal lesions showed decided evidence of regression or after they had healed completely.

Enlargement and induration of the inguinal lymph nodes in the rabbit were so constantly associated with scrotal infection that they could be used as diagnostic signs when any doubt existed as to the presence or absence of specific infection in the scrotum; not only this, but these signs could be so used before a scrotal lesion had had an opportunity to develop. The chief interest in involvement of the regional lymphatics, however, is their significance in connection with dissemination or generalization of the infection.

SUMMARY AND CONCLUSIONS.

From a study of the phenomena of the primary infection on the one hand, and the phenomena of local spread, or dissemination, on the other, it is seen that a multiplicity of lesions develops in the testicle and scrotum of the rabbit which have much the same characteristics irrespective of their origin. Some of these lesions are clearly recognizable as primary lesions or parts of a primary reaction to infection, while others are just as clearly the results of dissemination of the virus from a primary focus of infection or correspond with lesions which are commonly spoken of as secondary lesions. The effort to draw a sharp line of distinction between these two groups of lesions or between a primary and a secondary stage of infection in the rabbit, however, would be largely an arbitrary procedure. The fact is that the tissues of the scrotum and testicle of the rabbit constitute favorable surroundings for the localization and development of *pallidum* infections. Under ordinary circumstances, a large part of the reaction to infection which expresses itself in the formation of lesions recognizable by ordinary methods of examination takes place in these tissues. These lesions present certain broad and general characteristics without regard to whether they are primary or secondary in origin; the reaction is merely a reaction to a syphilitic infection which in either case may assume the most diverse character.

Further, it would appear that in rabbits infected with such strains of *Treponema pallidum* as we have used, the virus is never confined to the area occupied by the so called primary lesion, or chancre, but always spreads and always gives rise to a regional adenopathy. There may be no lesions to indicate the progress of this dissemination, but an examination of the inguinal nodes shows that dissemination occurs very soon after inoculation, and a *pallidum* reaction may be detected in these glands even before infection can be recognized in the scrotum. Subsequently lesions develop in all parts of the scrotum and testicle, sometimes involving the entire testicle or scrotum, and at others, forming focalized lesions with an especial predilection for certain locations such as the epididymis, the mediastinum testis, the tunics, and the dorsal folds of the scrotum. In some instances, more or less continuous lesions form along the course of the perivascular lymphatics, suggesting that this is one path taken in the dissemination of the organism. It is probable, however, that lesions of a gross character develop more as a result of accumulation of spirochetes than of mere invasion of the lymphatics since they are not a constant accompaniment of the local infection, while invasion of the lymphatics and extension of the infection to the regional lymph nodes occur in all cases.

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EXPLANATION OF PLATES.

The illustrations are all reproductions of untouched photographs which represent the objects at their natural size. Except where otherwise indicated, the statements of time are all estimated from the date of inoculation.

PLATE 90.

FIGS. 1 to 6. Lesions resulting from local extension, or local dissemination, in the scrotum.

FIG. 1. 85 days. Diffuse involvement of the scrotum with the formation of multiple focal lesions. The primary lesions are marked by arrows.

FIG. 2. Same animal. 91 days. Subsidence of the diffuse reaction with rapid evolution of the focal lesions.

FIG. 3. Same animal. 113 days. Final stage in the transformation of the disseminated lesions into a chancre-like lesion.

FIG. 4. 56 days. Perivascular lesions extending from the outer edge of an implant about which there has been very slight reaction. The implant is marked by an arrow.

FIG. 5. 63 days. A diffuse thickening of the scrotum with perivascular infiltrations along the vessels of the dorsal fold. The seat of the original chancres is indicated by the tiny crust on the right and the marked depression on the left.

FIG. 6. Same animal. 85 days. A later stage of the same lesions.

PLATE 91.

FIG. 7. 69 days. Multiple focal lesions of the scrotum with tendency to chancre-like transformations.

PLATE 92.

FIGS. 8 to 10. Recovery of incompletely healed chancres after treatment.

FIG. 8. 40 days. The chancres at the time of treatment.

FIG. 9. 2 weeks after treatment, marking the extent of the regression produced.

FIG. 10. 4 weeks after treatment. Compare the regeneration effected with the original lesions and with the extent of the regression produced in the two chancres.

FIGS. 11 to 13. Local recurrence after complete healing of scrotal chancres.

FIG. 11. 29 days. The chancres at the time of treatment.

FIG. 12. 2 weeks after treatment. The right chancre is healed, the left almost healed.

FIG. 13. 78 days after treatment. A recurrence in the form of multiple focal lesions which bear but little resemblance to the original chancres and only one of which could be regarded as a chancre recurrence.

PLATE 93.

FIGS. 14 to 16. Recurrence of focal and diffuse lesions after healing of chancres.

FIG. 14. 43 days. The chancres at the time of treatment.

FIG. 15. 35 days after treatment. The chancres completely healed.

FIG. 16. 64 days after treatment. Recurrence of multiple nodules with diffuse infiltration of the scrotum.

FIGS. 17 to 19. Recurrent papular lesions at the edge of the scar of a healed lesion.

FIG 17. 37 days. The chancres at the time of treatment.

FIG. 18. 21 days after treatment.

FIG. 19. 77 days after treatment. A thin, smooth scar with two small papules at its outer edge.

PLATE 94.

FIGS. 20 to 22. Healing of scrotal chancres with recurrence of chancre-like lesions entirely away from the site of the original lesions.

FIG. 20. 50 days. The original chancres.

FIG. 21. 7 days after treatment. Ulcers healed and chancres rapidly resolving.

FIG. 22. 133 days after treatment. Recurrent chancre-like lesion in the dorsal fold of the scrotum. The testicle is rotated towards the median line.

FIGS. 23 to 25. Transformation of a healing chancre into a diffuse infection of the scrotum followed later by multiple focal lesions of an erythematous character grouped mainly about the edges of the scar.

FIG. 23. 48 days. The time of treatment.

FIG. 24. 21 days after treatment. Chancres almost resolved but replaced by a diffuse infiltration of the scrotum. Note the thickened and refractile fold of the scrotum on the right.

FIG. 25. 35 days after treatment. Multiple focal lesions grouped particularly about the edges of the scars. Slight exfoliation.

PLATE 95.

FIGS. 26 to 28. Healing of scrotal chancres with recurrence in the head of the epididymis.

FIG. 26. 48 days. The chancres.

FIG. 27. 15 days after treatment. The chancres in process of healing.

FIG. 28. 30 days after treatment. The chancres healed; recurrent nodules in the head of the epididymis in both testicles.

FIGS. 29 to 31. Late recurrence of a minute papular lesion.

FIG. 29. 46 days. The chancres.

FIG. 30. 43 days after treatment. The chancres almost healed.

FIG. 31. 105 days after treatment. The recurrence. A minute point of infiltration in the scrotum, marked by the arrow.

PLATE 96.

FIGS. 32 to 37. Inguinal adenopathy associated with scrotal infection.

FIG. 32. Appearance of the inguinal region of a normal rabbit.

FIG. 33. 5 days. Acute swelling of inguinal nodes.

FIG. 34. 7 days. Acute swelling. Left, a single enlarged node; right, a large node situated over the cord and two smaller nodes at the edge of the ring, marked by arrows.

FIG. 35. 18 days. Enlargement with induration. Marked but unequal involvement of the nodes. Chancres small.

FIG. 36. 18 days. Marked lymphadenitis on the left with two small shotty nodes on the right, marked by arrows.

FIG. 37. 82 days. An asymmetrical involvement of the inguinal nodes. The enlarged node on the left is situated high in the groin.

PLATE 97.

FIGS. 38 to 43. Inguinal adenopathy associated with scrotal infection.

FIG. 38. 23 days. Lymphadenitis of moderate degree.

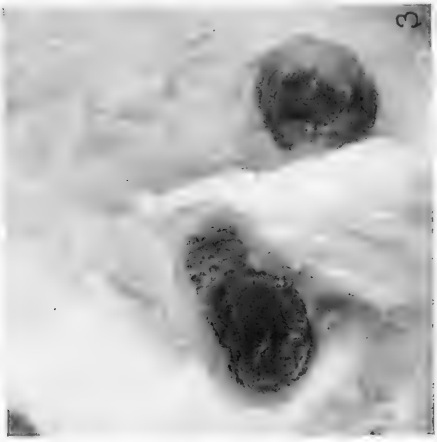
FIG. 39. 28 days. Indurated nodes. Chancres small; nodes large and unequal.

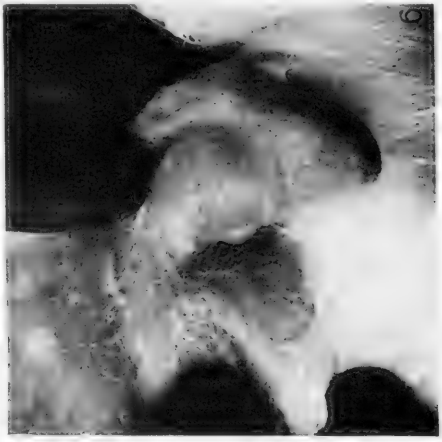
FIG. 40. 64 days. A flattened mass of small nodes on the right with a single rounded node on the left. Chancrés large; nodes moderately enlarged but intensely indurated.

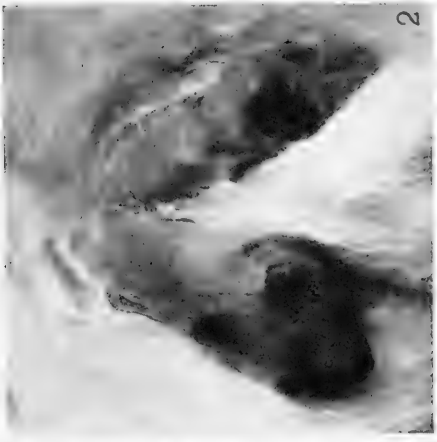
FIG. 41. 60 days. Extremely large chancres with apparently rather small lymph nodes. The nodes are deep, however, and lie directly in the crease of the groin.

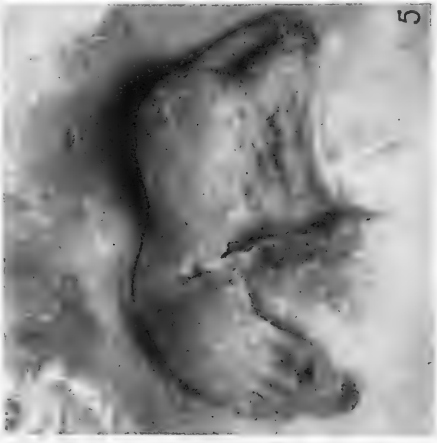
FIG. 42. Same as Fig. 41. The left node picked up between the thumb and finger.

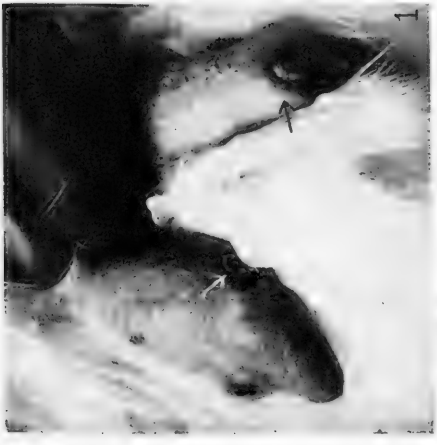
FIG. 43. 63 days. Marked equal and symmetrical lymphadenitis with slight lesions of the scrotum. Same animal as in Fig. 5.











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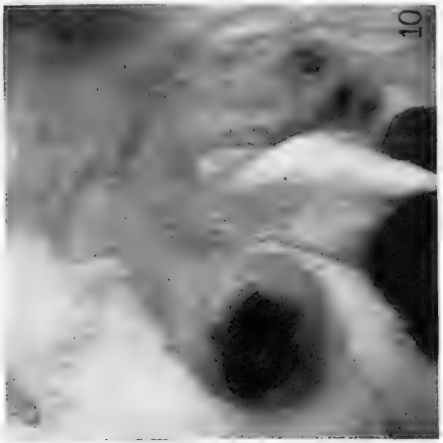

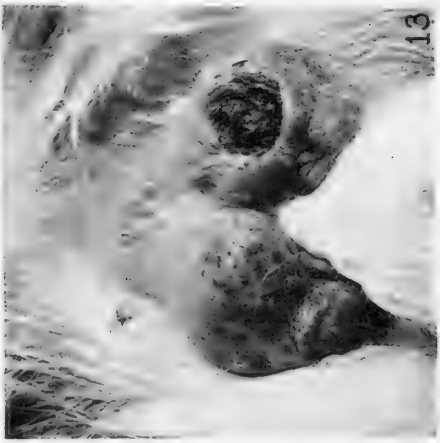

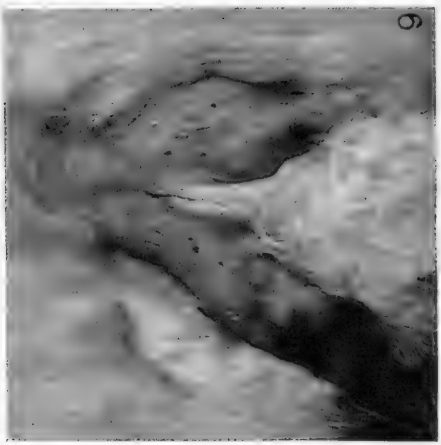

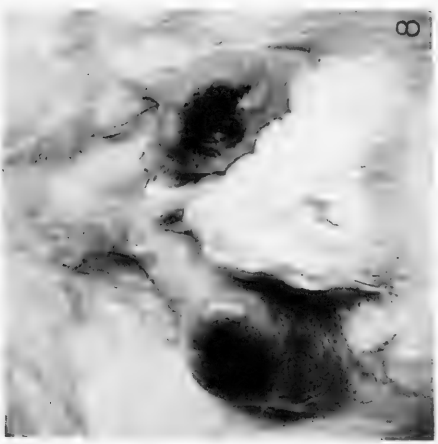

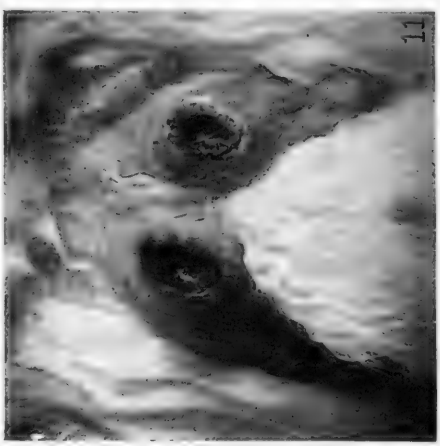

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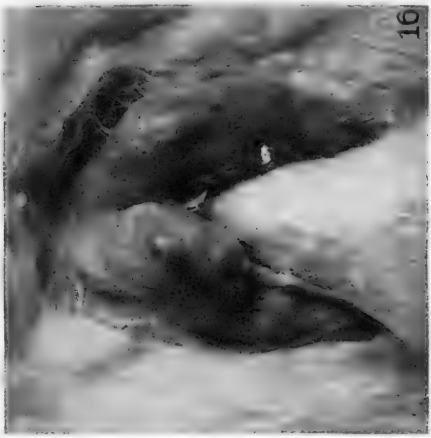

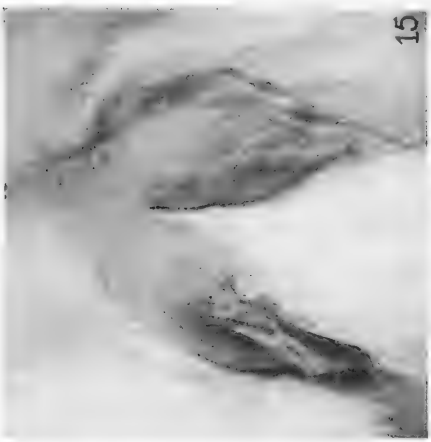

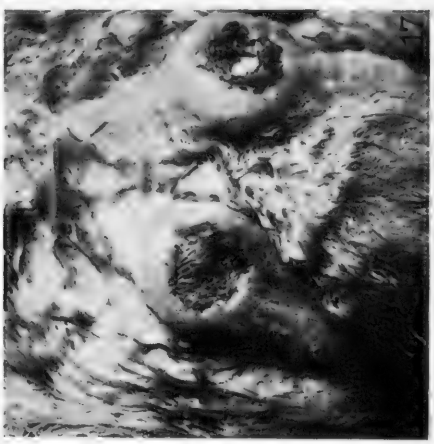

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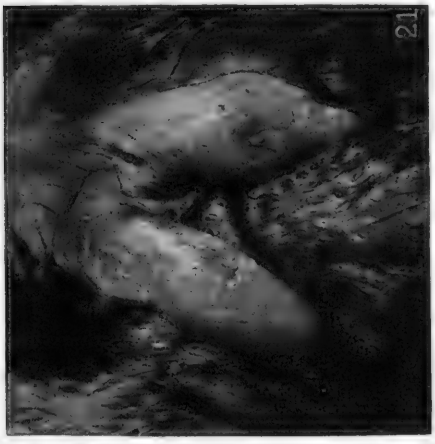

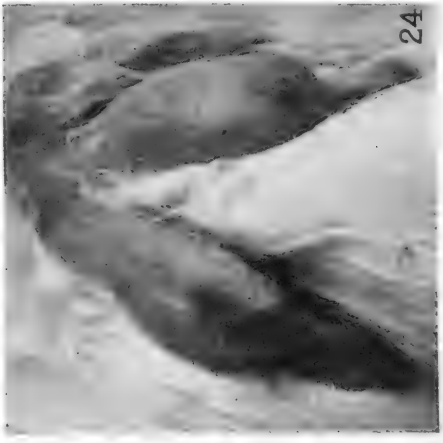

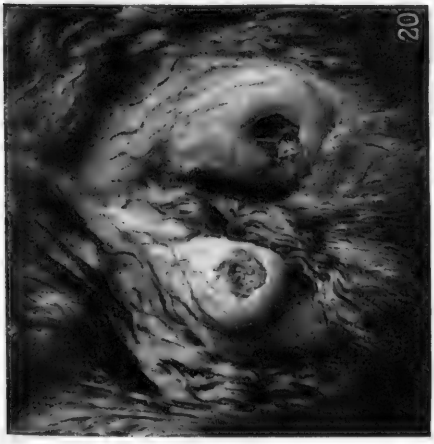

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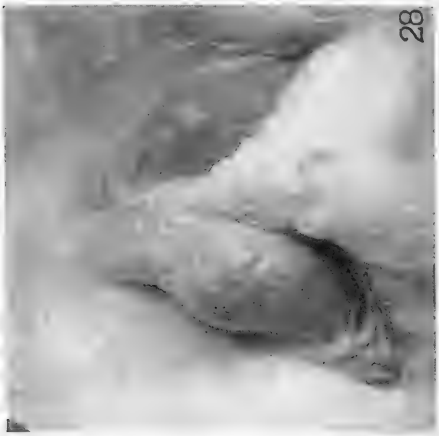

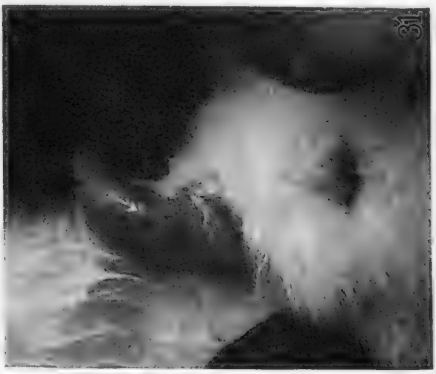

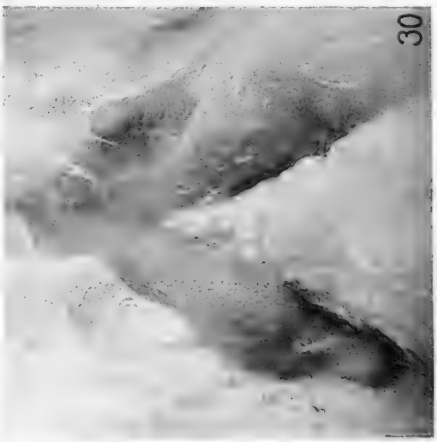

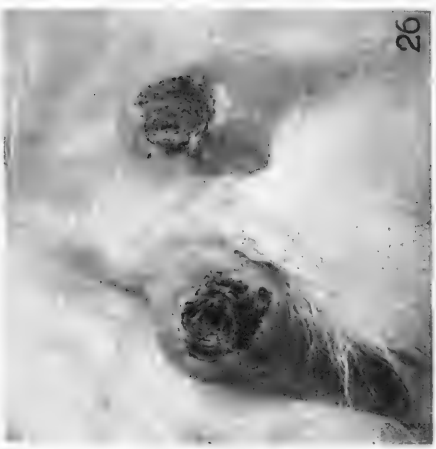

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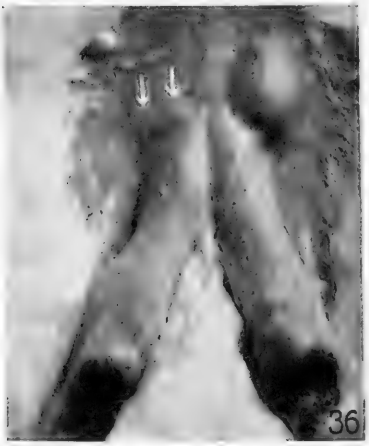

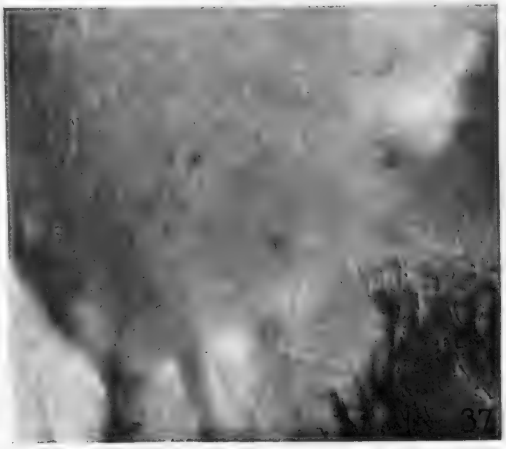

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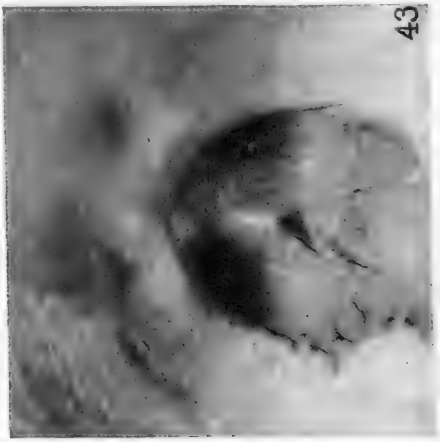

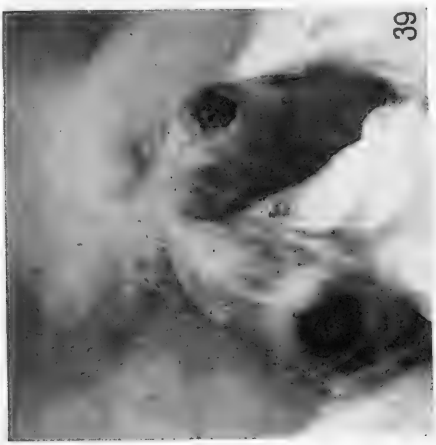

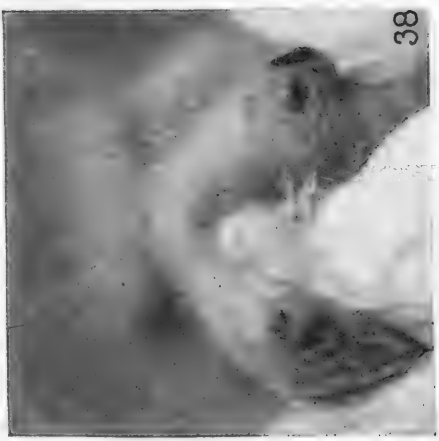

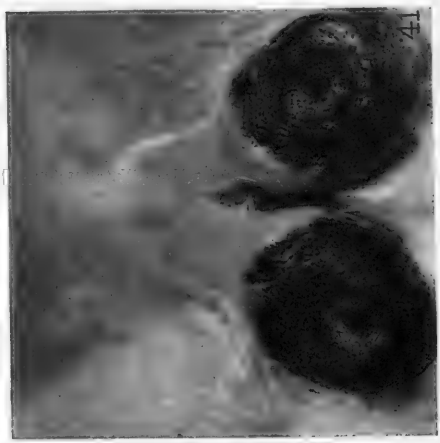

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HETEROTRANSPLANTATION OF THE THYROID GLAND.

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In two preceding communications the heterotransplantation of skin of the guinea pig[1] and of the pigeon[2] was discussed. At that time it was thought desirable to add to these studies others in which glandular tissues, free from bacteria, were used instead of skin, which is exposed to bacterial contamination. In this paper we deal with the heterotransplantation of the thyroid in mammals. This will be followed by a study of heterotransplantation of kidney tissue.

Our aim in these investigations is twofold. In the first place we wish to find the cause of the lack of success of heterotransplantation. And in the second place we use heterotransplantation as an additional method through which to analyze the laws underlying the reactions of various kinds of tissues towards each other.

Transplantation of the Thyroid Gland of the Guinea Pig into Rats.

Lobes of thyroid of guinea pigs were transplanted into twenty-four rats. In some instances only one, in others two lobes were transferred into each rat; occasionally a lobe of thyroid was transplanted into one side and a piece of kidney into the other. All pieces were placed into pockets of the subcutaneous tissue which had been made through a dorsal incision. The results were briefly as follows:

$3\frac{3}{4}$ *Days after Transplantation* (*One Experiment*).—The majority of acini are necrotic, and the acinus cells are pycnotic. In some places there are some better preserved acini, but with beginning degeneration (caryolysis). There are some fairly well preserved acini, with well preserved colloid. Only one mitosis is found in the transplant, and it is doubtful whether this mitosis is in an acinus cell.

[1] Loeb, Leo, and Addison, W. H. F., *Arch. Entwcklngsmechn. Organ.*, 1909, xxvii, 73.

[2] Loeb, Leo, and Addison, W. H. F., *Arch. Entwcklngsmechn. Organ.*, 1911, xxxii, 44.

Between and around the best acini a limited number of fibroblasts, some of which are hypertrophic, are found growing. But on the whole very little connective tissue grows between acini and into the necrotic center. In some places connective tissue cells grow between acini into the center without noticeable recovery of nearby degenerating acini. Few lymphocytes are visible, but in places connective tissue cells and some lymphocytes penetrate into the better preserved acini and may destroy some of them. No vessels are visible between the acini.

After 4 Days.—Many acini are relatively well preserved and a number of mitoses are seen in acinus cells. The well preserved acini are found where the surrounding connective tissue is more active and where some fibroblasts grow around and between acini. On the whole, few fibroblasts move between acini. Where the connective tissue is not active in the periphery of the transplant, the acini show somewhat shrunken nuclei. The center is necrotic, and little connective tissue is growing into it. In the periphery of the necrotic part some vessels are still preserved.

After 5 Days.—Several grafts were examined; they did not differ much from each other. The majority of the acini are not well preserved, but shrunken or in a state of caryorrhexis. In places there are better preserved acini, especially where a few better preserved fibroblasts are seen. The outer part of the parathyroid is preserved, the inner part necrotic. The connective tissue shows little activity. Only few fibroblasts grow around and between acini; they have a tendency to form fibrous tissue. Few vessels are near the transplant, but in certain places a few capillaries are found near by. Even directly around the capillaries the fibroblastic tissue becomes transformed into fibrous tissue. There are only few lymphocytes near acini, but in some places where vessels are around the transplant, there are many lymphocytes which may infiltrate some acini. This is, however, a comparatively rare finding. Lymphocytes are more frequently found where acini are missing. The center is necrotic. Some fibroblasts grow into the center and often degenerate here. Phagocytes take up some necrotic material.

After 6 Days.—Examination of several transplants showed essentially the same condition as in the previous stage. The number of well preserved acini is small. Their situation shows a relation to fibroblastic activity. In most places acini are shrunken and their cells vacuolar and caryolytic. There are in most pieces a few mitoses present in acinus cells, and some acini contain colloid. The connective tissue shows on the whole little activity. Where it does grow between acini, it has a tendency to form fibrous tissue. Only a small part of the central necrotic material is organized. Some capillaries grow around the transplant, and only rarely do they grow a short distance between some acini. In some places the lymphocytic infiltration in the neighborhood of the transplant is considerable, in others it is slight. Occasionally the number of lymphocytes around some acini is greater, and here they may even penetrate into the interior of some of the latter. This is found especially where a number of vessels are in the direct

neighborhood of the acini. Sometimes a few polynuclear leucocytes are seen in the transplant. In one animal the transplant has been entirely destroyed.

After 7 and 8 Days.—The transplants were found to be destroyed. Only fibrous tissue, some necrotic material, and lymphocytic infiltration were seen.

After 9 Days.—In three animals the transplants had been destroyed. A great part of the necrotic material had been organized. Some lymphocytic infiltration was visible. In a fourth and fifth rat some of the transplants were still alive. In the fourth animal there were still better preserved acini with a number of mitoses and large vesicular nuclei on one side of the transplant; but even here cells were somewhat vacuolar. A few, but not many connective tissue cells and lymphocytes were noted around the acini. In some acini retracted colloid was visible. In proliferating acini desquamating acinus cells had gotten into the colloid. In some acini (or remnants of ducts(?)) several rows of cells were formed. On the other side of the transplant acini were very vacuolar and degenerating; there was here no proliferation of connective tissue cells, and lymphocytes were absent. Especially noticeable is the absence of blood vessels between acini and in the center. The necrotic center is incompletely organized. In the fifth animal a small number of acini is still preserved. Some mitoses and some hypertrophic nuclei are seen in acinus cells. Dense fibrous tissue with few vessels compresses these acini; only few fibroblasts are between the acini. Some lymphocytes are around acini, and a few of these have gotten into the acini. There is no dense lymphocytic infiltration directly around the acini. Where on the other side a dense lymphocytic infiltration is present, acini are found to be lacking. The effect of compression by connective tissue is here more in evidence than the lymphocytic activity. Vessels are very scarce or absent between preserved acini.

9 days after transplantation was the latest date at which living thyroid tissue was still found. Following this period only connective tissue with or without some necrotic material and lymphocytes were observed. In some pieces infection had evidently taken place.

Conclusions.—As late as 9 days after transplantation a number of acini are preserved in two pieces and mitoses occur at this time. This is the latest term at which mitoses and living tissue are found. In general the greater part of the heterotransplanted thyroid suffered largely through the lack of suitability of the body fluids of the host. Many of the acini were degenerating in various ways. Evidently only those situated under especially favorable conditions were found better preserved. Wherever the acini were in a better condition the fibroblasts of the host showed greater activity near by; and conversely, where they were in a poor condition the fibroblasts were relatively inactive. But on the whole, the number of fibroblasts growing between the acini was relatively small, and the fibroblasts which did

grow around the acini had a tendency to form fibrous tissue. The blood vessel supply around and between the acini was very poor; but some capillaries penetrated here and there a little between some acini.

The lymphocytes were usually not numerous between and in the direct neighborhood of the acini. But in some cases they were more frequent at some distance from the transplant. Occasionally they extended from here into the transplant itself and invaded a few acini. Thus they did to a very limited extent contribute to the destruction of acini.

We see then that it is primarily an incompatibility between the body fluids and the transplant which is responsible for the small amount of surviving tissue. The destructive action of the lymphocytes and connective tissue plays only a minor part. The lymphocytes invade the transplant to a very slight extent. Vascularization and ingrowth of fibroblasts into the transplant are slight, much less so than in homoiotransplantation. In the latter there is also much more lymphocytic infiltration. Furthermore, the transplanted tissue is usually much better preserved and more active in homoiotransplantation.

Transplantation of the Thyroid Gland of the Rabbit into Rats.

After 3 Days.—The ring of acini is incomplete; it is interrupted, where muscle of the host forms the base of the graft. Elsewhere are well preserved acini with colloid. The acinus cells are succulent and seven to nine mitoses can be seen in acinus cells. Pycnotic nuclei disappear only in the periphery of the necrotic area. Fibroblasts move between the acini; some mitoses are visible in fibroblasts. Polynuclear leucocytes are found only in one place.

After 5 Days.—A well preserved ring of acini with retracted or vacuolar colloid is found. The cells of the acini are in good condition; they have on the whole a succulent character and the nuclei are well formed. Mitoses are, however, very rare in the acinus cells. Some fibroblasts and capillaries grow between the acini; but in general the amount of fibroblastic tissue proliferating around the acini is small. In one place, however, the fibroblasts are active and near by a mitosis is found in an acinus cell. When the acini are best preserved, most fibroblasts grow around them; here they make fibrous stroma. The center is not yet organized. Large cavities originate, probably through solution of necrotic tissue. In the connective tissue capsule there are lymphocytes which do not penetrate into the acinar zone. Polynuclear leucocytes are absent.

After 7 Days.—There is a well preserved ring of acini. Five mitoses were counted in acinus cells. The latter are of medium size and show vesicular nuclei. Some of the colloid is in good condition, other colloid is rarified or vacuolar. There are some cells in the colloid. Fibroblasts and a few capillaries penetrate between the acini. Only the peripheral part of the necrotic center is organized by a small amount of connective tissue. The zone of acini is surrounded by a fibrous capsule which is somewhat infiltrated with lymphocytes. The capsule with the lymphocytes is rather sharply separated from the thyroid ring. Few lymphocytes penetrate between acini and a very small number of polynuclear leucocytes is visible.

After 8 Days.—In this specimen conditions are in many respects similar to those in the preceding specimen, but there are certain differences. There are many well preserved acini, some of which are close together, and some mitoses are found in the acinus cells. The center is necrotic; here there is much infiltration with polynuclear leucocytes. These cells cause cavity formation in the peripheral parts of the necrotic material. A small part of the necrotic material is organized. Connective tissue surrounds and invades some acini; the fibroblasts form fibrillar tissue. Lymphocytes accompany the connective tissue, and both destroy some acini, the fibrous tissue compressing the acini. Very few vessels are visible and they enter the tissue mainly at places where thyroid acini are missing. Because of the lack of vessels which penetrate between acini, the lymphocytes attack the acini mainly from the outside, wherever such an attack occurs. In places connective tissue may grow around some acini without being associated with much lymphocytic infiltration; the fibroblasts produce fibrous tissue. The thyroid itself is not markedly infiltrated with lymphocytes. A connective tissue capsule surrounds the transplant.

After 9 Days.—There are well preserved acini close together with only exceptional mitoses. Some well preserved colloid is present in acini. The center is necrotic; it is only partly organized by fibrous tissue. Capillaries are found around the transplant, usually at some distance from the thyroid tissue. Here and there in the capsule may be large masses of lymphocytes; but in other places in the surrounding tissue lymphocytes are lacking around the capillaries. Lymphocytes enter the thyroid only exceptionally, but in a few cases they as well as fibroblasts destroy some acini. Although some capillaries enter the thyroid proper, still there is neither much growth of thyroid acini nor much lymphocytic infiltration in the transplant proper. Through a defect in the thyroid some vessels penetrate towards the center. Usually capillaries do not break through directly between the acini. Some fibroblasts penetrate between the acini and make fibrils; they produce fibrous tissue even in the direct neighborhood of the capillaries. The capsule around the graft is fairly fibrous.

After 11 Days.—In neither of the specimens of the first rat was living thyroid tissue present. In the first specimen some necrotic material as well as fibrous and fibroblastic tissue with capillaries and some lymphocytes were found. In the second specimen an epidermal cyst or its remnants were seen. In places connec-

tive tissue was growing into the keratin and formed epithelioid cells around the particles of keratin. In certain areas polynuclear leucocytes separated the epidermis from the underlying connective tissue; in such places the epidermis was necrotic. In the periphery at a distance from the transplant there was a ring of lymphocytes. This epidermal cyst originated probably in a thyroid duct, such as was found to occur in the thyroid in the other specimens.

In a second rat some thyroid was preserved after 11 days. There were groups of small acini surrounded as a whole and individually by band-like fibrous tissue. No mitoses are found in acinus cells, the latter of which are on the whole low or cuboidal; some remnants of vacuolar colloid are present in the lumen of the acini. No large masses of lymphocytes invade them. There are small collections of lymphocytes separated from acini by fibrous bands. In places, however, some isolated lymphocytes infiltrate acini and help to destroy them. Some fibroblasts may likewise invade and destroy a few acini. Certain acinus cells are becoming vacuolar and degenerate. In the surrounding fibrous tissue quite a number of scattered polynuclears are visible which occasionally may infiltrate the acini and help to destroy them. In the fibrous tissue there is a large duct with stratified epithelium. Some epithelial cells desquamate, and some polynuclear leucocytes penetrate into the duct lumen. From this duct strands of epithelial tissue radiate; they are surrounded by fibrous tissue but are not usually invaded by lymphocytes or polynuclear leucocytes. Occasionally, however, some lymphocytes or polynuclears invade them and some of the former may also invade the stratified duct. Exceptionally, some lymphocytes around these cell strands may destroy part of them. The epithelial cells are evidently not resistant; they are near degeneration and fall an easy prey to various kinds of attacking cells such as polynuclear leucocytes, lymphocytes, and fibroblasts.

Around the ring of acini there is a concentric capsule of fibrous tissue. At the outside of the fibrous tissue we find collections of lymphocytes and some polynuclear leucocytes. These peripheral masses of lymphocytes are situated around capillaries. In the fibrillar tissue which surrounds the acini there are some capillaries, but usually no lymphocytes. Some parts of the central necrotic tissue are organized, other parts are as yet in process of organization. Two stages are found in the organization of necrotic material: (1) a hyaline substance is produced through mixture of the cytoplasm of the advancing fibroblasts and necrotic material; and (2) a fibroblastic tissue with many capillaries and sometimes lymphocytes is secondarily produced.

After 12 Days.—Here again merely stratified epithelium is found which is partly alive, partly necrotic. In the surrounding fibrous tissue there is much lymphocytic infiltration.

After 13, 14, and 15 Days.—No living thyroid tissue was found, but only some remnants of necrotic tissue, fibrous tissue, vessels, and lymphocytes. The same two strata of organizing material are observed; namely, (1) a lamellated, hyaline, avascular zone, and (2) infiltrating this a cellular, vascular, lymphocytic tissue.

Conclusions.—Living acini were found as late as 11 days after transplantation. This occurred in one of three pieces. Mitoses were found not later than 9 days after transplantation; but at that time they were exceptional. At 11 and 12 days epidermal ducts or cysts were still seen in cases in which the acini had already perished. In one instance, 11 days after transplantation, the epidermal duct had given origin to strands of squamous epithelium. This indicates that the squamous epithelium is more resistant to the injurious action of heterotoxins than the thyroid acini. Similarly we found previously that the squamous epithelium of the kidney pelvis may be more resistant to homoiotoxins than the gland tissue.

Under the influence of the heterotoxins the epithelial cells degenerate directly, and in addition, they are less resistant to the action of lymphocytes, polynuclear leucocytes, and fibroblasts. They are easily affected by various kinds of injurious influences. While fibroblasts now and then penetrate between the acini, there is, on the whole, not much fibroblastic ingrowth into the thyroid; the fibroblasts however, that do grow around the acini have a tendency to form fibrous tissue. Capillaries may grow actively in the tissue which surrounds the transplant, but they do not usually grow between the acini, although in places an ingrowth of capillaries occurs. Lymphocytes may be present in large masses around the transplant near the vessels, but only here and there do they penetrate between the acini, and they then help to destroy some of the latter. Even in the neighborhood of capillaries which grow close to, or in the interstices between acini the lymphocytes are scarce. The presence of capillaries does not prevent the formation of fibrous tissue from fibroblasts; it occurs even in the direct neighborhood of the vessels.

The thyroid of the rabbit transplanted into the rat is well preserved at a relatively late stage, namely 9 days after transplantation; accordingly, mitoses are more frequent up to the 9th day than in the other kinds of heterotransplantation; but at 11 days some degenerative processes have become noticeable.

Transplantation of the Thyroid Gland of the Rabbit into Guinea Pigs.

After 3 Days.—An excellent ring of living thyroid preserved. Very rare mitoses in acinus cells. Some well formed colloid in acini. No degeneration of cells. Center necrotic. In the center of the necrotic area pycnotic nuclei are preserved, while in the periphery they have been lost. There are a few scattered polynuclear leucocytes. Some fibroblasts and a few capillaries growing between thyroid acini. Some parathyroid and transplanted fat tissue preserved.

After 6 Days.—Ring of thyroid tissue here and there preserved, but on the whole only a small part is still alive and this is not in good condition. The cells do not stain well or the tissue shows signs of shrinking. The cells are not succulent and only exceptional mitoses are seen. Some acini have colloid, others while close together are without lumen or colloid. In places capillaries enter between the acini. Some connective tissue, a few lymphocytes, and a relatively considerable number of scattered polynuclear leucocytes grow into the transplant and help to destroy it. Small celled connective tissue penetrates into the poorly preserved part, compresses it, and penetrates occasionally into the acini. Lymphocytes approach the acini from the neighborhood of the vessels and enter and destroy some acini. The number of polynuclear leucocytes between the acini is relatively large. In some places masses of polynuclear leucocytes destroy the tissue. The necrotic center is in process of organization by connective tissue. The transplanted thyroid tissue is not vigorous, but near the point of death; migrating cells contribute to this destruction.

After 7 Days.—The thyroid is necrotic. In the center pycnotic nuclei are preserved, in the periphery they are lost. Connective tissue is growing in the periphery of the necrotic piece. There is no lymphocytic infiltration.

After 8 Days.—Well preserved acini, close together with and without colloid; also larger ducts present; no mitoses. In certain areas of the thyroid ring are large acini with well preserved cells and nuclei.

A thick capsule of dense fibrous tissue with few vessels around the thyroid. Where there is a defect in the thyroid ring, many capillaries enter the transplant together with connective tissue; where the thyroid ring is preserved, only few vessels can be found. On the whole, the vascularization of the thyroid is very poor. Some connective tissue penetrates between acini, and fibroblasts form here a thin stroma; they do not usually destroy acini. Only small parts of the necrotic material in the center of the transplant are organized. In the center of the necrotic material there are still some pycnotic nuclei, while in the periphery they have been destroyed. Polynuclear leucocytes collect in the surrounding capsule; they penetrate into the thyroid and destroy some of the acini. They also migrate into the necrotic center and dissolve a part of the necrotic material. There are some lymphocytes in the surrounding connective tissue, but there is not much lymphocytic infiltration in and around acini. There are large areas of preserved thyroid without lymphocytic infiltration. In certain places, however, lymphocytes are so numerous that they destroy acini.

After 10, 11, and 12 Days.—The thyroid was no longer preserved. There was some necrotic material with some polynuclear leucocytes. There is slight lymphocytic infiltration. In one specimen parts of the necrotic material have become dissolved.

Conclusions.—After transplantation of the thyroid of the rabbit into guinea pigs the results were not so satisfactory as after transplantation into rats. Well preserved thyroid was found as late as 8 days after transplantation, but not at later dates. The last mitoses were observed 6 days after transplantation and at that time they were few in number. Otherwise the conditions are similar to those generally seen in heterotransplantations. The ingrowth of vessels is slight. Fibroblasts likewise do not grow in very actively and have a tendency to produce fibrous tissue. The capsule surrounding the graft is very fibrous. Here lymphocytes are in places present in large numbers; but they penetrate into the thyroid only to a limited extent. The distribution of the lymphocytes in the surrounding tissue depends upon the vessels. Lymphocytes and fibroblasts may here and there contribute to the destruction of acini. These transplantations differ from those in the rat in the greater number of polynuclear leucocytes which are usually found in the guinea pig. They may be seen scattered in the surrounding capsule, and from here they penetrate into the thyroid proper and help to destroy some acini. They also may enter necrotic material and dissolve some parts of it. We are inclined to assume that the inferiority of the results in this series is due to the activity of the polynuclear leucocytes or to the conditions which call forth their appearance. This difference between transplantation into the rat and guinea pig may be caused by the fact that the transplantation of tissue into dorsal pockets in the rat insures sterility of the tissue to a greater extent than transplantation into ventral pockets as practised in the guinea pig. It is possible that after heterotransplantation the transplants offer better soil for the action of microorganisms than after autotransplantation or homoiotransplantation.

Transplantation of the Thyroid Gland of the Cat into Rats.

After 3 Days.—Well preserved thyroid ring. The acini are close together and have colloid. There are medium sized cells in good condition, but some cells are slightly vacuolar. No mitoses. Fibroblasts and a number of capillaries coming

from capillaries in the surrounding tissue grow between acini. No lymphocytes. There is little organization in the necrotic center. The central part of the necrotic material is with, the periphery without pycnotic nuclei. On the whole, the piece is well preserved but the acinus cells are not succulent. In places a few polynuclear leucocytes migrate through the thyroid ring into the necrotic center.

After 4 Days.—Well preserved ring of thyroid with apparently healthy, but not succulent cells; occasionally vacuolar cells. Some acini with retracted colloid, many acini with very small lumen and little colloid. No mitoses. The central part of the necrotic material is with, the periphery without pycnotic nuclei. A few fibroblasts grow between the acini and a small number of polynuclear leucocytes wanders through the thyroid ring into the center. Occasional small collections of lymphocytes around peripheral vessels, but no marked lymphocytic infiltration of the transplant. Blood vessels and probably also lymph vessels grow from the outside into the center through the thyroid; they probably are connected with the old preexisting vessels in the graft. Even in the neighborhood of the blood vessels no mitoses in acinus cells.

After 5 Days.—Well preserved ring of thyroid; acini with colloid. Only exceptional mitoses in acinus cells; even where there is much fibroblastic tissue around acini, there are usually no mitoses. No succulent acinus cells. In the fibroblastic tissue which surrounds the transplant many blood vessels are seen and occasionally some collections of lymphocytes around vessels. A vessel grows from the outside into septa of the thyroid; in this case probably a transplanted vessel connects with neighboring vessels. Where there is much fibroblastic tissue around the transplant, fibroblasts and some vessels may grow around acini. Fibroblasts with some lymphocytes may separate, and occasionally penetrate into acini. Usually lymphocytes are absent around acini. In the periphery of the piece we find cavities; they are either lymph spaces or dissolved necrotic tissue.

After 6 Days.—There is not so much thyroid tissue preserved as in some of the previous specimens, but on one side of the piece a zone of acini is present. In some acini there is retracted colloid, in others it is lost. Mitoses are lacking. Around the transplant there is fibroblastic tissue with many capillaries. Where there is much fibroblastic growth in the neighborhood of the acini, some vessels, fibroblasts, and lymphocytes penetrate between, and some lymphocytes penetrate even into the acini. Fibroblasts provide a stroma between some acini. On the whole, there are few lymphocytes around or in the thyroid. Only isolated connective tissue strands are growing into the necrotic center. In places there are large spaces around the thyroid.

After 7 Days.—Well preserved but incomplete thyroid ring. Acini close together and with colloid which is somewhat vacuolar. The nuclei of the acinus cells are often a little deformed, and some vacuoles are found around them. Fibroblasts and capillaries proliferate around grafts. In places capillaries grow plentifully from the outside in between acini; yet mitoses in acinus cells are absent and there is not much lymphocytic infiltration around acini. Fibroblasts fur-

nish some stroma for the acini, growing between them. Here some mitoses in fibroblasts may be found. In the surrounding fibroblastic tissue there is in places a moderate infiltration with lymphocytes. Lymphocytes may also penetrate between acini and destroy a few of them, but the infiltration is not extensive, being much less marked than after homoioplastic transplantation. Some polynuclear leucocytes are in the necrotic material where thyroid is lacking.

After 8 Days.—An incomplete ring of fairly well preserved acini with colloid. Some acinus cells are vacuolar and the nuclei irregular; in general acini healthy but not turgescent. The acini form islands in the periphery of the graft. Where fibroblasts and vessels are found near the thyroid, they may penetrate into the transplant. Connective tissue furnishes a stroma for the thyroid; while it has organized some necrotic material, most of the latter is as yet unorganized. In places connective tissue cells and a few lymphocytes penetrate between and into acini and destroy some, but not many of them. In such places we may find some mitoses which are probably of fibroblastic origin. There is no real lymphocytic infiltration. Some large lymph vessels grow into the center of the thyroid and adjoin directly the living thyroid. Notwithstanding this fact, there is not much lymphocytic infiltration. Some parathyroid is also preserved.

After 9 Days.—The results obtained with two pieces are similar. There is an incomplete ring of thyroid tissue. The acini are in many places well preserved and may contain retracted colloid or be without lumen; many acini are close together. In other places, especially where fibrous tissue surrounds and compresses the acinus cells, some of the latter are vacuolar and the nuclei deformed. There are some, but not many mitoses in acinus cells. Where fibroblasts penetrate into acini and destroy them, mitoses are found, but whether these are in acinus cells or fibroblasts is uncertain. A few capillaries are found around acini, but the presence of vessels does not call forth cell proliferation. On the whole, the connective tissue proliferation between the acini is not marked. From the surrounding connective tissue capsule connective tissue grows between acini, surrounds some of them, and chokes them, thus contributing to their destruction. These acini may disintegrate. In other places fibroblasts proliferate around acini; here the latter are relatively better preserved. But fibroblasts may also penetrate into some acini and destroy them. There is no noticeable lymphocytic infiltration. Lymphocytes are found in the surrounding capsule and with the fibroblastic tissue they may penetrate between some acini and help to destroy some of them. However, they do not play a significant part and are less important than the connective tissue so far as the destruction of acini is concerned; but even the connective tissue is not very active as a destructive agent. The necrotic center is only partly organized. Connective tissue penetrates into it, especially where the thyroid ring is interrupted. Through solution of the necrotic material cavities are formed which are not unlike lymph spaces.

At one place in the connective tissue, where the thyroid ring is interrupted, squamous cell ducts are found in the connective tissue; structures not unlike keratin pearls are formed from these ducts.

After 10 Days.—Small areas of acini are left; some acini are close together; there is still colloid present in some of them. On the whole, acinus cells are not well preserved; there is much vacuolation, especially where fibrous tissue compresses acini. Usually living acini prevent connective tissue from organizing necrotic material. Elsewhere connective tissue grows into the necrotic center which has as yet been only partly organized. Strands of fibroblastic-fibrillar tissue with some lymphocytes get near the thyroid acini, but usually stop here. Occasionally, however, connective tissue and some lymphocytes penetrate into acini and help to destroy them, but this is not a frequent occurrence. Where fibroblasts and some lymphocytes invade acini, mitoses are found, perhaps in acinus cells. Wherever there is fibroblastic proliferation near the thyroid, some lymphocytes may penetrate into adjoining acini and help to destroy them. In some places in the surrounding connective tissue there is much lymphocytic infiltration. The connective tissue growing around acini forms occasionally fibrous bands which may choke acini which thus become vacuolar. In another animal the findings are similar, but the acini are at least in part fairly well preserved; there are numerous acini with some colloid. A few mitoses and hypertrophic nuclei occur in acini. In other places, however, the acini become vacuolar and their nuclei pycnotic. Some acini are close together; others are surrounded by fibrous tissue. There is no dense lymphocytic infiltration in, or directly around the acini, but a few lymphocytes and connective tissue cells seem to migrate into some acini. The connective tissue around the acini is fibrous or fibrillar. In places such fibrillar-fibroblastic tissue encircles acini and penetrates between them. Some fibroblasts grow around the better preserved acini, but usually the connective tissue around the acini is fibrous. In such acini degenerative processes may set in. Whole acini may become pycnotic; other cells become vacuolar. Migration of lymphocytes and small connective tissue cells, if it takes place at all, is isolated; in the surrounding fibrous capsule, on the other hand, the lymphocytic infiltration may be considerable. There are a few mitoses in acinus cells. On the whole, the vascularization is very poor; in places, however, there is vascular tissue with many capillaries around the thyroid, and here may be found some lymphocytes. At one place a vessel with some lymphocytes penetrates between the acini. There are frequent mitoses either in fibroblasts or endothelium of blood vessels and the connective tissue penetrates into the central necrotic tissue especially where the thyroid ring is interrupted. The tissue supplanting the necrotic material is dense.

After 11 Days.—Peripheral thyroid tissue is preserved, but many acinus cells show signs of degeneration, especially vacuolation. Acini situated in fibrous bands may become transformed into small cell nests without colloid. Other acini, however, still retain some colloid. A few mitoses are observed in acinus cells, but their number is smaller than in homoiotransplants at the corresponding stage. The stroma is usually fibrillar or fibrous and shows very poor vascularization. Connective tissue cells frequently break into the ring of acini and into the acini themselves and destroy them. Also a few, but not many lymphocytes may

penetrate between and into acini. Usually the connective tissue forms fibrils or fibrous bands around acini, thus choking them. The connective tissue penetrates also through the ring of acini to the inner aspect of it and attacks it from the inside. Here again it forms fibrous tissue. The thyroid ring exerts a certain, though slight restraining influence on the connective tissue. Thus the thyroid to a certain extent inhibits the rapid organization of the central necrotic material. This organization proceeds more rapidly where the thyroid ring is absent, because here connective tissue can penetrate more readily into the necrotic material. Thus it comes about that the central necrotic material may adjoin directly the thyroid ring. Under the conditions of heterotransplantation the thyroid still has some restraining influence on the connective tissue, but it is very much restricted. Furthermore, the epithelial elements under the influence of the heterotoxins show little power of resistance to injury. The constricting effect of the fibrous tissue, the absence of good vascularization, and the direct attacks by fibroblasts and lymphocytes, though limited, all contribute to the destruction of the graft. These destructive factors are not counterbalanced by a new production of tissue sufficient to make good the constant loss. In the necrotic material polynuclear leucocytes produce in certain places a solution of the material.

After 12 Days.—In the first animal only little thyroid tissue is preserved; considerable parts of the thyroid are degenerating. Peripheral acini become vacuolar and the stroma between them also degenerates in places. There are visible areas of collapsed acini without colloid. The degenerating parts of thyroid are replaced by fibrous tissue. Some connective tissue cells and lymphocytes migrate into the degenerating areas. Fibrous tissue, poor in cells, surrounds acini. Only little lymphocytic infiltration. There are, however, still small bundles of acini infiltrated by some lymphocytes; there is in places much lymphocytic infiltration in fibrous tissue around and at some distance from the thyroid. Here the tissue may be rich in fibroblasts and blood vessels. Much necrotic tissue is organized, especially where the thyroid is interrupted. The organization takes place in two stages. In the first stage much necrotic tissue is organized by avascular connective tissue; secondarily this tissue becomes organized by fibroblastic, lymphocytic tissue with blood vessels. At one place in the necrotic material there is an abscess (localized infection). In another animal no living thyroid was found in two pieces, only necrotic material, fibrous tissue, and some lymphocytic infiltration.

After 14 Days.—Conditions are similar. Many acini are in process of degeneration; they show vacuolation or are surrounded by dense fibrous tissue and are small, evidently compressed, and without lumen and colloid. In other acini colloid is found. Mitoses in acinus cells are absent. Vascularization is poor, but a few capillaries are found between acini. The connective tissue has a tendency to form dense fibrous encircling bands around acini, but in some places it is still richer in cells and less fibrous, and thus it penetrates between acini. Such connective tissue pushes into some acini and may destroy them. Wherever the connective tissue is still more fibroblastic, some lymphocytes are usually found

in it which may enter acini and contribute to their destruction. But on the whole the lymphocytic infiltration is slight, and even where lymphocytes are near the thyroid, they usually do not enter the thyroid proper; they do not seem to be attracted by the heterologous tissue. The fibroblastic character of the connective tissue is present only in certain places, and only occasionally is a mitosis found in a fibroblast between acini. Soon this connective tissue becomes converted into fibrous tissue very poor in cells. Connective tissue penetrates also into the necrotic center, mainly at places where the thyroid ring is interrupted. The thyroid acini present a certain obstacle to the advance of the connective tissue. The necrotic tissue is therefore least organized where it adjoins directly the thyroid. The organization of the necrotic tissue takes place in two phases. First a hyaline avascular tissue is produced which is later substituted by fibroblastic vascular tissue.

In a second animal a greater part of the transplant was dead 14 days after transplantation. The majority of the acini which have not yet become necrotic are dying. They are vacuolar and the nuclei are deformed or have disappeared. There are still some acini with vesicular nuclei. Where all the tissue is necrotic the peripheral parts are organized by connective tissue and blood vessels. Near such blood vessels there are considerable masses of lymphocytes. These lymphocytes, however, do not usually penetrate into the living, vacuolar thyroid tissue. The acini are often surrounded by avascular fibrous tissue. A few connective tissue cells may penetrate between the dying transplanted acini and surround some of them. Around the transplant there are in places some empty spaces, probably the result of the solution of necrotic material. Such spaces were also found in the second animal at this period.

The tissue is injured primarily as a direct result of heterotoxin action. The behavior of fibroblasts and blood vessels and to some extent the action of lymphocytes contribute to the destructive result.

After 15 Days.—No living thyroid left. A small amount of unorganized necrotic material was still present. The greater part of it had been changed into fibrous tissue around which there was much lymphocytic infiltration. In the necrotic material there were some giant cells.

After 16 Days.—No living thyroid found. In the periphery there are some necrotic acini with shrunken cells and nuclei. A part of the peripheral necrotic tissue is organized and substituted by fibrous tissue. The graft is surrounded by dense fibrous tissue with very few vessels. Here there is in places marked lymphocytic infiltration. In the necrotic transplant itself no lymphocytes are found. Some empty spaces are seen, similar to those described above. They may represent lymph spaces or dissolved fat tissue. There are some epithelioid and small giant cells around the necrotic part. In the fat disintegrating polynuclear leucocytes are found.

After 18 Days.—The transplant has become entirely necrotic with the exception of a few very vacuolar acini in the periphery. The nuclei in these acini are mostly deformed. There is no marked infiltration by lymphocytes or connective tissue

cells. No blood vessels can be seen between the acini which are still left in the periphery. A part of the necrotic material has been organized by connective tissue. As usual the organization takes place in two stages. At first a hyaline tissue is formed in which vessels are lacking. It represents a substitution of necrotic material by fibroplasm or a mixture of the two. Later a cellular connective tissue with lymphocytes supplants it. Around the graft there is dense fibrous tissue with many lymphocytes.

Conclusions.—At an early stage following transplantation the thyroid tissue of the cat is fairly well preserved and colloid may be found in the acini; but at no time does the transplanted parenchyma have a succulent character, such as may be observed after autotransplantation or even after homoiotransplantation. As early as 5 days after transplantation an occasional invasion of an acinus by fibroblasts and lymphocytes and a subsequent destruction of these acini are found. These conditions prevailed from the 3rd to the 7th day after transplantation. After 7 days some signs of beginning degeneration appear in some acinus cells, such cells being vacuolar. Many acini are, however, well preserved. At 8 days vacuolation is also noticeable. At 9 days degeneration is distinct in some places. Fibrous tissue surrounds and compresses some acini. Other acini are destroyed by invading fibroblasts and lymphocytes. This is, however, not a prominent feature of the process. After 10 and 11 days the destructive effect of compression by fibrous tissue is still more marked. Whole acini become vacuolar or even pycnotic. On the whole, conditions are similar to those found after 9 days, but perhaps more marked. After 12 days there is much degeneration of the thyroid. The amount of preserved tissue is relatively small. Vacuolation and collapse of acini are noticeable. After 14 days the same vacuolation and compression of acini are found. There is much degeneration. Fibroblasts and lymphocytes enter some acini and destroy them. Especially in one piece do we find much vacuolation, deformation, and loss of nuclei; a great part of the transplant is entirely necrotic, other parts are dying. However, some relatively well preserved acini can still be found. At later periods mainly necrotic tissue is present. Some acini may be shrunken, other thyroid tissue is entirely dead. But as late as 18 days following transplantation there may be present a small amount of degenerating thyroid tissue which has not yet become entirely necrotic.

The first mitoses were seen 5 days after transplantation. After homoiotransplantation mitoses appear at earlier stages. There is, therefore, from the beginning a depression in mitotic proliferation after heterotransplantation. This inhibition is quite marked also in the following period; 6, 7, and 8 days after transplantation no definite mitoses were found. However, 9, 10, and 11 days following heterotransplantation a limited number of mitoses appeared. 11 days was the latest date at which they were found. The heterotoxins produce, therefore, a marked diminution in the number of mitoses in the transplanted thyroid.

The character of the vascularization of the heterotransplants is of great importance. 3 and 4 days after transplantation capillaries are found in the thyroid between the acinus cells. At 5 and 6 days fibroblastic tissue with capillaries can be seen around the grafts. From here a few vessels may penetrate between acini. Occasionally there is a specimen in which in certain places the vascularization between the acini is more plentiful. Such was the case in a specimen taken out 7 days after transplantation. From 8 to 14 days after transplantation there are usually a few capillaries between acini, but on the whole the lack of a satisfactory vascularization is quite apparent. The stroma is characterized by its lack of a good blood vessel supply.

The behavior of the connective tissue towards the heterotransplants is a determining factor in the result of the transplantation. At early stages, 5 and 6 days after transplantation, we find fibroblastic tissue with capillaries around the graft; some fibroblasts enter between the acini at various places and supply a stroma. Wherever fibroblasts are growing in larger number between the acini, they may enter the latter and destroy them. This is the condition found in the first 8 days. Mitoses are only rarely seen in the fibroblasts. In the following period, from the 9th to the 15th day, fibroblasts occasionally penetrating between and into acini are still found, but the fibrous character of the connective tissue around the acini is much more marked. This is, indeed, a noticeable effect of heterotransplantation. The stroma is, on the whole, densely fibrous or fibrillar and poor in vessels. This stroma compresses a considerable number of acini and exerts an injurious effect upon them. While fibroblasts thus

continue to break into the thyroid at various places, this infiltration of the interacinar spaces is in general not very extensive so long as there is living thyroid tissue present. Even under conditions of heterotransplantation the thyroid tissue exerts a certain inhibiting influence on the connective tissue of the host; the inhibition on the part of the parenchyma is greater than that presented by necrotic tissue. Thus the organization of the necrotic center is mainly effected by connective tissue growing into it at places where the thyroid ring is incomplete, while at places where the necrotic material adjoins the inner aspect of the thyroid the organization is much delayed.

Marked infiltration of the transplants by lymphocytes is not seen. Wherever there is much fibroblastic proliferation around the transplant and between the acini there may be small numbers of lymphocytes accompanying the fibroblasts. Towards the latter part of the 1st week and during the 2nd week lymphocytes may participate in the destruction of acini to a limited degree. In the 2nd week marked lymphocytic infiltration in the surrounding capsule at some distance from the thyroid proper may occasionally be found; lymphocytes may also sometimes penetrate into some adjoining furrows reaching into the transplant. But a marked collection of lymphocytes around and in the thyroid acini, such as is characteristic of homoiotransplantation, is not observed in heterotransplants. Even where the supply of blood vessels between the acini happens to be somewhat more extensive, a marked lymphocytic infiltration is not observed.

Lymphocytic infiltration may also be somewhat more marked in the dense fibrous tissue which is the result of the organization of necrotic material. Here again living thyroid tissue is absent. This organization of necrotic material takes place in two stages, the definite organization by a vascular connective tissue rich in fibroblasts and lymphocytes being preceded by the provisional production of hyaline material, representing a mixture of necrotic material and fibroplasm.

Ducts lined by squamous cell epithelium may temporarily survive after heterotransplantation.

In conclusion we find that the heterotoxins injure the transplanted cells directly and make them thereby susceptible to additional injurious influences, such as lack of good vascularization, fibrous character of the stroma causing compression of acini, and direct invasion and

destruction of a limited number of acini by fibroblasts and lymphocytes. These factors lead to a constant dying of transplanted parenchyma which is not compensated for by any appreciable new formation of tissue. The primary factor in the destruction of the heterotransplant is the injurious effect of the heterotoxins. They change the metabolism of the transplanted cells which now exert an effect upon the blood vessels, connective tissue cells, and lymphocytes which differs in certain respects from that exerted by normal as well as auto- and homoiotransplanted cells. These secondary reactions contribute to the destruction of the heterotransplants.

SUMMARY.

1. After transplantation of the thyroid of the guinea pig into rats there is a primary injury of the transplant, noticeable as early as 3, 4, and 5 days after transplantation. The tissue is less resistant, and it is preserved only under the best conditions. The number of mitoses is much diminished in the transplant, but they may appear even as late as 9 days after transplantation. This was also the latest time at which living tissue was found. Epithelium is best preserved in the neighborhood of growing fibroblastic tissue, and growing epithelium attracts fibroblasts. Few fibroblasts grow between acini, and they have a tendency to form fibrous tissue. Dense fibrous tissue compresses acini and contributes to their destruction. The vascularization of the graft is very poor, but some capillaries may penetrate between acini. The collection of lymphocytes around acini is only casual and may be found where fibroblasts are active and especially around the blood vessels in the capsule of the graft. On the whole, heterotransplanted tissue does not attract lymphocytes to any marked extent. Connective tissue and lymphocytes contribute only secondarily and to a minor extent to the destruction of the heterotransplant.

2. After transplantation of the thyroid of the rabbit into rats the last mitoses are found 9 days after transplantation; living acini are observed as late as 11 days after transplantation, and ducts of squamous epithelium even somewhat later. Different kinds of tissue seem to show a different degree of resistance to the action of hetero-

toxins. The difference in the resistance to heterotoxins corresponds to the difference in the resistance of various tissues to other kinds of injurious influences. The thyroid of rabbits is, on the whole, better preserved in the rat than the thyroid of the guinea pig; there is also more mitotic activity in the rabbit thyroid.

The reaction of the tissues of the host towards heterotransplanted rabbit thyroid in principle is similar to the reaction to heterotransplanted guinea pig thyroid.

3. After transplantation of rabbit thyroid into guinea pigs well preserved thyroid is found not later than 8 days, while the last mitoses appear 6 days after transplantation. The number of mitoses is very small. The host tissues again behave in a manner characteristic of heterotransplantation. It is probable that slight infections and the presence of polynuclear leucocytes are responsible for the somewhat inferior results in this kind of heterotransplantation.

4. After transplantation of cat thyroid into the rat, signs of degeneration in the transplanted acini appear at the end of the 1st week; they increase during the 2nd week. The last well preserved acini are found at 14 days; a few degenerating acini are still visible as late as 18 days after transplantation. Almost all pieces are entirely necrotic in the 3rd week. Mitoses are only found 5, 9, 10, and 11 days after transplantation, and they are present in a limited number. There is a decided lack of good vascularization in the transplants; it is especially noticeable in the 2nd week after transplantation. Fibroblasts penetrate at various places between the acini and occasionally together with a few lymphocytes may destroy some of them. From the 9th day on the presence of fibrous tissue around the acini is noticeable. It compresses the acini and thus contributes to their destruction. Even the heterotransplanted thyroid exhibits a restraining influence on the connective tissue which is greater than that presented by dead material. The behavior of the lymphocytes towards heterotransplanted thyroid of the cat is similar to that towards other heterotransplanted thyroid.

CONCLUSIONS.

After heterotransplantation of thyroid of various mammalian species into the rat and guinea pig, the latest period at which living tissue is found varies between 8 and 14 days. Exceptionally dying tissue may be found somewhat later. The last mitoses are observed between the 9th and 11th days in cases in which the tissue is found completely necrotic between the 9th and 14th days, and on the 6th day when the tissue is found dead 8 days after transplantation.

The heterotoxins injure the transplanted tissue directly and primarily and thus make it more susceptible to additional injurious influences, such as lack of good vascularization, fibrous character of the stroma, and direct invasion and destruction of a limited number of acini by fibroblasts and lymphocytes. Especially noticeable is the slight reaction of the lymphoctes towards the heterotransplants as compared with their strong reaction towards homoiotransplants.

A constant destruction of heterotransplanted parenchyma is not compensated for by any marked new formation of tissue. The action of the heterotoxins changes the metabolism of the transplanted tissue in such a way that the reaction of the various host tissues (vessels, fibroblasts, lymphocytes) towards the transplanted parenchyma is altered.

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